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ISSN 2575-0305

Journal of Molecular Medicine and Clinical Applications

Research Article | Volume: 1.1 | Open Access

Regulation of Egr1 by Mtor/P70s6k Pathway is Associated with Cognitive Decline in Ovariectomized Mice

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Received date: 12 Dec 2015; **Accepted date:** 02 Jan 2016; **Published date:** 07 Jan 2016.

Citation: Zhang S, Cong J, Zhou W, We J (2016) Regulation of Egr1 by Mtor/P70s6k Pathway is Associated with Cognitive Decline in Ovariectomized Mice. J Mol Med Clin Appl 1(1): doi http://dx.doi.org/10.16966/2575-0305.101

Abstract

Background: To study the association between mTOR/p70S6K signaling pathway and early growth response1 (Egr1) in regulating cognitive decline of ovariectomized (OVX) mice.

Methods: Using the Morris water maze (MWM) and electro-physiological examination, we assessed the changes of cognition function in OVX-mice and Sham. The expression of Egr1, mTOR, and p70S6K in hippocampus was detected before and after intra-cerebroventricularly rapamycin-treatment in OVX and Sham groups by quantitative real-time polymerase chain reaction and Western blot. Besides, expression of mTOR and p70S6K was monitored in SH-SY5Y cells following transfection with Egr1 interference fragment or Egr1-overexpressing plasmid.

Results: Escape latency was significantly increased; time in target platform quadrant was reduced, while synaptic plasticity was damaged in OVX-mice compared with Sham. Moreover, the hippocampal Egr1, mTOR and p70S6K expression levels were obviously increased at OVX-mice compared with Sham. After rapamycin treatment, expression of mTOR/ p70S6K and Egr1 were significantly down-regulated in OVX mice. Additionally, in SH-SY5Y cells, either the Egr1 interference fragment or the Egr1-overexpressing plasmid transfection did not alter the level of mTOR and p70S6K expression.

Conclusions: Our data suggest that increasing Egr1 expression, relating to cognitive decline in OVX, is regulated by mTOR/ p70S6K signaling. Interesting, mTOR inhibitor-rapamycin, reducing the Egr1 level, might be a potential therapeutic target in postmenopausal cognitive decline.

Keywords: Cognitive impairment; Early growth response 1; mTOR/p70S6K signaling pathway; Ovariectomy; Rapamycin

Introduction

Mild cognitive impairment (MCI) is the early clinical stage of Alzheimer's disease (AD) and it's characterized by impairment in learning and memory [1,2]. Nowadays, more than 20% people over 80 years old of age are affected by AD. Epidemiological data predict that over 35 millions of people will be affected by 2050 over world [3], which will cause heavy social and economic burden. So far, limited benefits have been obtained from available therapeutic agents to slow down this disease progression.

Menopause women, as the decline of estrogen level, are susceptible to cognitive impairment [4,5]. Ovariectomy in female rodents is a widely recognized model to mimic postmenopausal pathophysiological changes in humans [6,7]. Our previous results [8] and other experimental data [9] demonstrate that decreased estrogen levels in ovariectomized rodents are associated with decreased brain activity overall and poor memory, especially hippocampus-dependent learning [10]. As researchers Orawan et al. once reported that ovariectomized mice not only decreased serum 17-estradiol level and uterine weight, but also impaired object recognition performance in the novel object recognition test and spatial cognitive performance in the Y-maze test and the water maze test [11].

Early growth response 1 (Egr1) belongs to the zinc finger family of transcriptional factors [12,13], which participates in a variety of mechanisms mediating growth, proliferation, differentiation and apoptosis [14-16]. Recently, researches have shown that the Egr1 changes attribute to the progression of memory deficit [17,18]. Gersten et al. studied that Egr1, as a key molecule in hippocampus-related learning and memory is down-regulated in simian immunodeficiency virus-infected hippocampus, leading to deficits in cognition [19]. Interestingly, our recent study showed that Egr1 messenger RNA (mRNA) and protein level were elevated in ovariectomized mice, which were linked to their cognitive impairment [20].

mTOR belongs to a family of specific serine/threonine protein kinases. Protein mTOR exists in two mTOR protein complexes mTORC1 and mTORC2 with various sensitivity to the inhibitory effect of rapamycin [21]. p70S6K (the serine/threonine kinase ribosomal protein) is one of the major downstream targets regulated by mTOR activity [22]. In recent years, more and more studies have shown that the mTOR signaling pathway is closely related with cognition deficiency [23,24]. Besides, overwhelming evidence showed that the elevated activity of mTOR signaling pathway has been found in AD patients [25,26]. Caccamo et al. have reported that suppressing mTOR signaling could rescue the memory deficits in Tg2576 mice that are widely used animal model of AD [27]. Mechanistically, the reduction in mTOR signaling could restore the hippocampal gene expression signature [28]. These results implicate that hyperactive mTOR signaling may represent a molecular pathway by which aging contributes to the development of AD.

Interestingly, the abnormal expression of Egr1 and mTOR/p70S6K signaling respectively has relationship with cognition deficits. Whether there is potential association between the over-expression of Egr1 and

mTOR/p70S6K signaling activity remains to be illuminated. Therefore, in the present study, we investigated the changes of the mTOR/p70S6K signaling pathway and cognition function in OVX mice, and further clarified the potential relationship between Egr1 expression and the mTOR/p70S6K pathway. Through our study, we hope to provide clues for the prevention and treatment of cognitive impairment of postmenopausal women in the future clinical practice.

Materials and Methods

Animals and surgery

Female ICR mice (Medical Animal Center of Nanjing Medical University, Jiangsu Province, China), weighed 25-30 g (8-12 weeks) at the beginning of the experiment, were used throughout the study. All animals were housed under a standard light/dark cycle at a mean (SEM) constant temperature of 23°C (±1° C). Mice (n=50) were anesthetized with chloral hydrate (500 mg/kg IP) and randomly divided into two groups to undergo either bilateral ovariectomy (n=25) or sham operation (n=25) by the ventral approach using sterile surgical techniques. Visual inspection corroborated the complete resection of the ovaries. Before and after all procedures, five mice were housed in a plastic hanging cage and permitted free access to food and tap water. The study was approved by the Animal and Human Ethics Board of the First Affiliated Hospital, Nanjing Medical University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Morris water maze test

Morris water maze (MWM) test was performed to test learning and memory in mice. It was divided into four quadrants: southeast, southwest, northeast, and northwest, with a 10 cm black circular escape platform placed at a fixed location, approximately 2 cm under the water surface. Exposed at 25°C room temperature, the water was opaque and the pool was coated with black non-toxic tempura paint. Mice were trained to find the hidden platform for 5 consecutive days, four training trials per day. If the mouse reached the platform within 60 s, it was allowed to stay on it for 10s; otherwise, it was gently guided to the platform and remains on it for 10s. Time taken to reach the platform (escape latency) and spent in the target quadrant was recorded by a video tracking system during probe tests.

Drug administration

An intracerebroventricular injection was performed through a stereotaxic apparatus. Mice were anesthetized with 2% chloral hydrate (20 ml/kg) and placed in a stereotactic device (Kopf Instruments, Tujunga, CA). The injection site was confirmed in preliminary experiments by injecting Indian ink. A 26-gauge single guide cannula (Plastics One, Roanoke, VA) was implanted into the right lateral ventricle (0.3 mm posterior, 1.0 mm lateral, and 2.5 mm ventral to bregma). After surgery, a 28-gauge dummy cannula (Plastics One, Roanoke, VA) was inserted into each guide cannula. Neither insertion of the needle nor injection of the saline had a significant influence on survival, and behavioral responses or cognitive functions.

Rapamycin was dissolved in dimethylsulfoxide (DMSO, Sigma, St Louis, MO, USA) and then in saline solution, final concentration being 20 mg/kg. DMSO was used at a final concentration of 2% as vehicle control. The drugs were injected with a stepper-motorized microsyringe (Stoelting, Wood Dale, IL, USA) at a rate of 0.5 ml/min (final volume 0.6 µl/mouse). Rapamycin (1.0 mg/kg) or equal volume vehicle control was delivered gradually over the course of 2 min.

Cell culture and treatment

SH-SY5Y neuroblastoma cell line (ATCC, Shanghai, China) is a very popular cell model to clarify the molecular mechanisms of Alzheimer disease in many researches [29,30]. SH-SY5Y Cells were cultivated in Dulbecco's modified Eagle's medium/F12 supplemented with 10% heat-inactivated fetal bovine serum (Tianhang, Hangzhou, China) in a humidified atmosphere containing 5% CO2 at 37˚C. To evaluate the relationship between Egr1 and mTOR/p70S6K signaling pathway, we transfected SH-SY5Y cells with Egr1-overexpressing plasmid (GV141-Egr1 plasmid) , GV141-Egr1 control (vector), Egr1 siRNA and si-Egr1 control (si-Egr1 NC) (Genechem, Shanghai, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA). Besides, the mTOR inhibitor rapamycin (4 µM) was applied to the cells to further conform the results. Cells were collected for total RNA isolation or protein purification at 48 hours after transfection or rapamycin treatment.

RNA extraction and quantitative real-time PCR

Homogenization of the fresh hippocampus tissues and isolation of total RNA were performed according to the manufacturer's instructions using a Trizol-based commercial kit (Takara Shuzo Co. Ltd., Kyoto, Japan). The purity of each RNA sample was determined by the absorbance ratio at 260 and 280 nm. The integrity of RNA preparations was evaluated by electrophoresis on a 1.2% (w/v) agarose gel containing 0.005% (v/v) a nucleic acid dye, Goldview (Shanghai SaiBaiSheng, Shanghai, China). The extracted RNA, containing ribosomal 28S and 18S RNA with a ratio of absorbance intensity 1.0–1.5 was used for qRT-PCR. In the real-time PCR reaction, cDNAs were used as templates for amplification to quantify the mRNA levels of target genes by using Quantitect SYBR Green PCR kits (Takara Shuzo Co Ltd, Kyoto, Japan). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6 was used as an internal control for sample loading and normalization. The comparative Ct (threshold cycle) method with arithmetic formulas ($2^{-\Delta\Delta Ct}$) was used to determine the relative amount of mRNA.

Western blot analysis

Total protein samples were homogenized in RIPA buffer containing protease inhibitors and phosphatase inhibitors. Proteins from all extractions were quantified using a BCA Protein Assay Kit (KC-430; Kang Chen). The protein samples were separated by 8% sodium dodecyl sufate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The membranes were blocked with 5% nonfat milk or 5% bovine serum albumin in Tris-buffered saline+1% Tween 20(TBST) for 1 hour at 37˚C. Then membranes were incubated with the appropriate primary antibodies: rabbit anti phospho-mTOR (p-mTOR) antibody (1:1000, Ab109268; Abcam, London, UK), rabbit anti-mTOR antibody (1:1000, Ab87540; Abcam), rabbit anti phospho-p70S6K (p-p70S6K) antibody (1:1000, Ab109393; Abcam), rabbit anti-p70S6K antibody (1:1000, Ab32359; Abcam), rabbit anti-Egr1 antibody (1:5000, ab194357; Abcam), and rabbit anti-tubulin antibody (1:5000, ab176560; Abcam) overnight at 4°C. After five washes with TBST for 10 minutes at 37°C, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Beijing ZhongShan, Beijing, China) for 1 hour at 37°C. Bands were detected using an ECL detection kit (Amercontrol Biosciences,London, UK).

Immunofluorescence and confocal imaging

To evaluate intracellular expression levels of egr1 and mTOR by using immunofluorescence and confocal microscopy, SH-SY5Y cells were cultured on poly-d-lysine coated coverslips for 24 h. Cells plated on coverslips were then fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min, washed in PBS one time, permeabilized in 0.1% Triton X-100, and blocked in 1% BSA, 50 mM glycine and 2% normal serum. The primary antibodies against the following proteins were used: rabbit anti-mTOR antibody (1:100), rabbit anti-Egr1 antibody (1:100), for overnight at 4°C. On the next day, coverslips were washed with PBS

and then incubated with the FITC (fluorescein isothiocyanate) marked secondary antibodies and applied for 1 h at room temperature. To stain the nuclei,1 μg/ml(w/v) 4′, 6-diamidino-2-phenylindole (DAPI, Sigma, USA) was added for 5 min. Confocal immunofluorescence images (1024×1024 pixels) were acquired on the Olympus Fluoview 1000 laser scanning confocal microscope using a 20x and 40x objective with numerical aperture 1.42.

Electrophysiological analysis

Brains were quickly removed and placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124NaCl, $2CaCl_2$, 4.5KCl, 1.0 $MgCl_2$, 26$NaHCO_3$, 1.2NaH_2PO_4, and 10D-glucose and adjusted to pH7.4 by bubbling with 95% O_2/5% CO_2 mixture. Coronal brain slices (400-mm-thick) were cut using a vibrating microtome (Microslicer DTK 1500, Dousaka EM Co, Kyoto, Japan) in ice-cold oxygenated (95% O_2/5%CO_2) ACSF.

For recording, the slices were transferred to a chamber continuously perfused with oxygenated ACSF (2ml/min) maintained at 30˚C. Stimulation-evoked population spike (PS) were recorded from the BLA by a glass micropipettes filled with 2 M NaCl (4-5 MΩ) connected to an Axoclamp2B amplifier (Axon Instruments, Foster City, CA, USA). PS response was sampled using pCLAMP software (Axon Instruments, Foster City, CA, USA). Input-output (I/O) curve was built by plotting excitatory post-synaptic potential (EPSP) slopes against delivering stimulation intensities from 0.1 mA to 1.1 mA that ranged from sub-threshold intensity for elicitation of a PS to those eliciting maximal responses. To induce long-term change, a single train of high frequency stimulation (HFS) with 50% of maximal stimulus strength at 100 Hz for 1 s duration was delivered. To evaluate long-term potentiation (LTP), the same recording as that pre-HFS continued for 60 min post-HFS and the data were expressed as the percentage of the mean pre-HFS value. The successful LTP induction requires the increase of PS amplitude post-HFS during the stable phase (>30 min post-HFS) exceeds a minimum of 20%.

Statistical analysis

The data were shown as the mean ± standard deviation and the results were analyzed using one-way ANOVA test together with the Scheffe' multiple-range test by the SPSS statistical package 20.0 (SPSS Inc., Chicago, IL, USA). The one-way ANOVA test was used to evaluate generally the difference among groups and the Scheffe' multiple-range test was used to compare the two groups. $P<0.05$ was considered as statistical significance.

Results

Changes of cognitive behavior in ovariectomized 12-week mice

During the training, all mice spent less time to find the hidden platform day by day, but the 12-week-old OVX mice significantly show a longer escape latency than the age-matched sham-op mice in the last day of training trials ($P<0.05$, n=25; Figure 1A). Compared with Sham mice, the 12-week-old OVX mice spent less time in the quadrant that the hidden platform located before ($P<0.01$; Figure 1B). As shown in Figure 1C, the exploring trait typical swimming patterns in Sham mice were characterized by an obviously longer distance in target quadrant, whereas concentric swimming paths representing reduced platform crossings were found in OVX mice.

Influence of OVX on hippocampal LTP induction

To explore the effects of ovariectomy on neurons, we investigated the electrophysiological changes of perforant path-granule cell synapses. As shown in Figure 1D, the EPSP slopes in ovariectomized 12 week-mice were consistently smaller than that in the age-matched Sham mice at the stimulation of 0.1-1.1 mA ($P<0.05$; n=6). HFS can induce a significant increased EPSP in perforant path-granule cell synapses lasting for at least 60 minutes (which is named as LTP) in the hippocampus of Sham mice. However, similar HFS cannot result in LTP induction in ovariectomized mice (Figure 1E).

Expression of Egr1 and mTOR pathway in mouse hippocampus

The expression levels of Egr1 mRNA and Egr1 protein in the hippocampus were significantly increased in 12-week-old OVX mice compared with the Sham group ($P<0.01$; Figure 2C). Notably, we found that mRNA level of mTOR/p70S6K signaling pathway also upregulated in OVX mice. As shown in Figures 2A and 2B, the expression of mTOR and p70S6K mRNA were 1.45 fold and 1.38 fold higher respectively in the hippocampus at 12 weeks after ovariectomy compared with sham-operated mice ($P<0.05$). Besides, our results suggest that not only total protein of mTOR and p70S6K upregulated in OVX mice, phospho-mTOR at Ser2448 and phospho-p70S6K at Ser371 protein were significantly higher than Sham mice ($P<0.05$; Figure 2G).

To explore the relationship between Egr1 and mTOR signaling pathway, OVX mice after MWM test were treated with the mTOR inhibitor rapamycin (1.0 mg/kg) for a week via intra-cerebroventricular injection (i.c.v). In comparison to vehicle control, the rapamycin treatment negatively regulated Egr1 mRNA expression ($P<0.01$; Figure 2F), as well as mTOR signaling pathway ($P<0.01$; Figures 2D and 2E). Results of protein changes were consistent with mRNA analysis ($P<0.05$; Figure 2H).

Association of Egr1 expression and mTOR signaling pathway in SH-SY5Y cells

Through immunofluorescence and confocal microscopy, the subcellular localization of Egr1 and mTOR in SH-SY5Y cells were evaluated. The subcellular localization of Egr1 was mainly in perinuclear cytoplasm and nuclei (Figure 3A), while mTOR was mainly observed in perinuclear cytoplasm (Figure 3B). Next, we transfected cells with the si-Egr1 fragment or the Egr1 overexpressing plasmid to explore the effect of Egr1 on mTOR/p70S6K expression. 48 hours after transfection with Egr1-overexpressing plasmid or si-Egr1 fragment, mTOR and p70S6K expression in SH-SY5Y cells were not changed compared with the control group, including the mRNA and protein levels ($P>0.05$; Figures 4A-4H). To further confirm the association of Egr1 and mTOR/p70S6K, cells were finally treated with rapamycin (4 μM) for 48 hours. Consistent with the above results in hippocampus of OVX mice, rapamycin treatment negatively regulated Egr1 mRNA expression ($P<0.01$; Figure 5C), as well as mTOR/p70S6K signaling pathway ($P<0.01$, $P<0.05$; Figures 5A and 5B). There was similar results in protein levels after rapamycin treatment ($P<0.05$; Fiure 5D).

Discussion

In this study, we confirmed that learning and memory may be impaired by ovariectomy at 12 weeks mice, including the increased escape latency, reduced time in target platform quadrant through MWM test, which is similar to our previous results [31]. Moreover, we found that there was remarkable difference of electrophysiological examination in OVX and Sham mice, in that EPSP slopes in ovariectomized mice were consistently smaller than in the Sham mice and the deficit of LTP induction in OVX was occurred. Generally, EPSP slopes and LTP induction were used to evaluate synaptic plasticity of neurons, which is closely related with cognition function [32,33]. According to many studies, estrogen can dramatically increase hippocampal dendritic spine and synapse density and its protective function in cognition has been well discovered in recent years [34,35]. Thus, estrogen absence in mice 12 weeks after ovariectomy could impair synaptic plasticity in our research, and finally leading to cognitive decline. These results were supported by some other studies concerning the electrophysiological changes after reducing estrogen level in mammalian model [36,37].

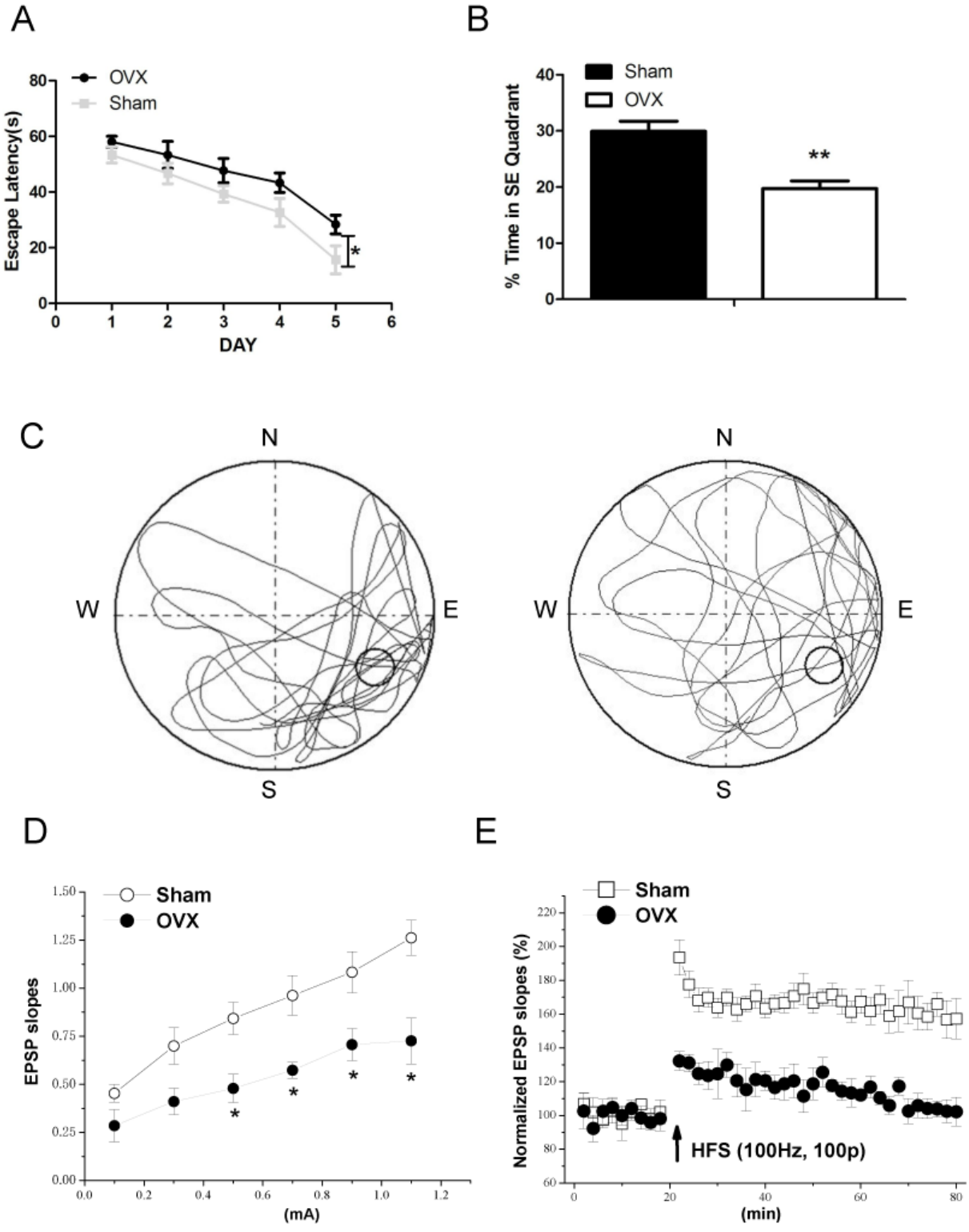

Figure 1: Changes in cognitive function at 12 weeks after ovariectomy

A: Comparison of escape latency in finding the platform in three other nontarget quadrants between ovariectomized (OVX) and sham-operated (Sham) mice. **B:** Time spent swimming in the target quadrant in the probe trial during 5 days of training in Morris water maze (MWM) for each group. **C:** Representative path tracings of the probe test on day 6 of MWM for each group. **D:** Input-output (I/O) curve. EPSP slope was evoked by perforant path-stimuli with a current from 0.1 to 1.1 mA. Each point represents group mean value (SEM) of EPSP slope. **E:** Changes of path-granule cell synaptic transmission in OVX and Sham mice slices with induction of LTP by 100 Hz-CS. *P<0.05, **P<0.01.

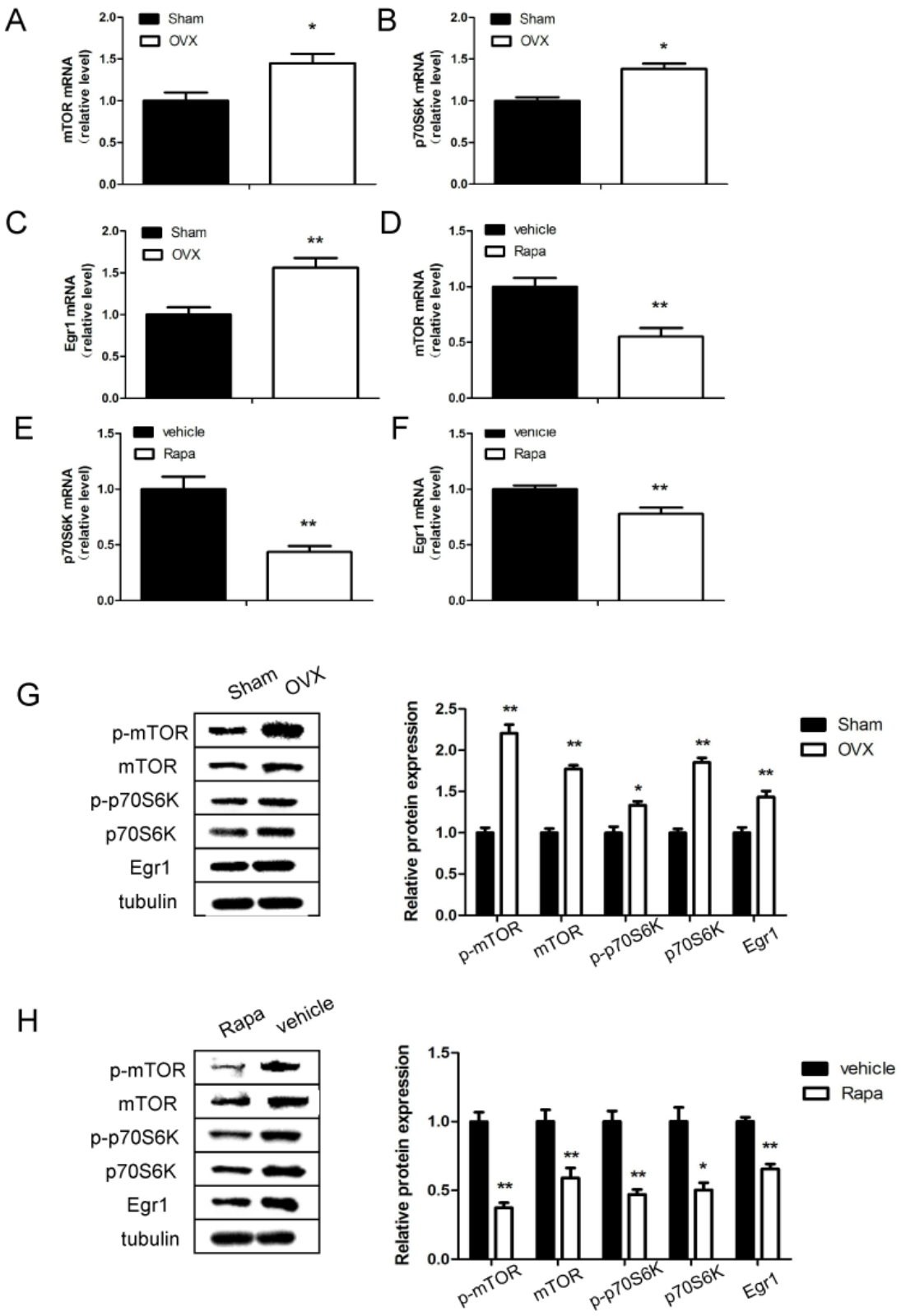

Figure 2: Expression of Egr1, mTOR and p70S6K in the hippocampus of ovariectomized (OVX) and sham-operated (Sham) mice

Quantitative reverse transcription polymerase chain reaction analysis of messenger RNA levels of mTOR (**A** and **D**), p70S6K (**B** and **E**) and Egr1 (**C** and **F**) in ovariectomized and rapamycin treatment mice respectively. **G:** Western blot analysis of protein levels of Egr1, mTOR and p70S6K 12 weeks after ovariectomy. **H:** Relative protein levels of Egr1, mTOR and p70S6K after rapamycin treatment for a week. *P<0.05, **P<0.01.

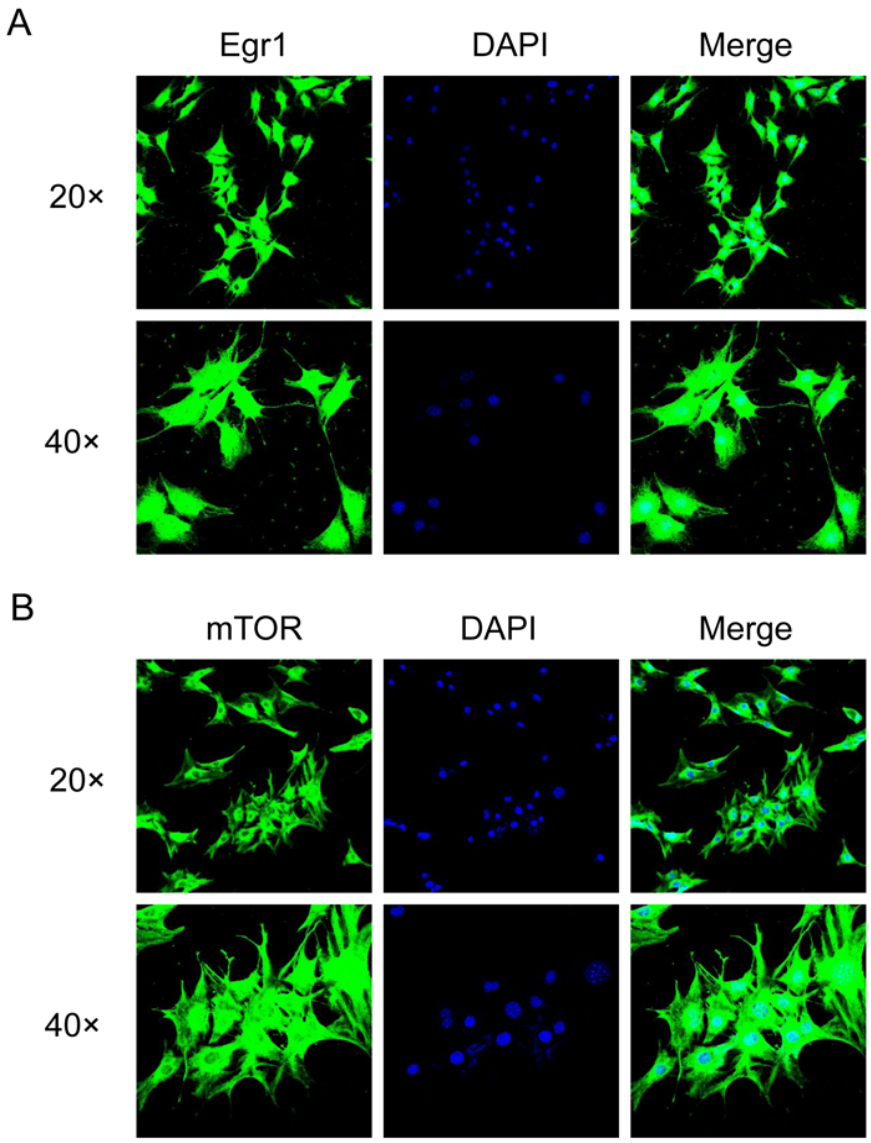

Figure 3: Confocal immunoflorescence microscopy of subcellular localization of Egr1 and mTOR in SH-SY5Y cells.
A: subcellular localization of Egr1 protein. **B:** subcellular localization of mTOR protein. Cells were seeded onto coverslips and stained respectively with Egr1 and mTOR antibodies, then the FITC (green) marked secondary antibodies. The nuclei were visualized using 4′, 6-diamidino-2-phenylindole (DAPI, blue).

Egr1 has been well described as a participant in hippocampus-related learning and memory. It plays an essential role in the maintenance and formation of LTP, and adult neurogenesis as shown by its role in the selection, maturation, and functional integration of dentate gyrus newborn neurons [38]. Similar to our previous reported, the over-expression of Egr1 was determined in the hippocampus at 12 weeks after ovariectomy in our recent study. However, since there are also reports showing downregulation of Egr1 in simian hippocampus, leading to deficits in cognition [19], further studies on the determination of Egr1 expression in different species and cells should be performed to elucidate these discrepancies. Interestingly, our present study also shown that when cognition deficiency occurs in mice 12 weeks after ovariectomy, the levels of mTOR, phospho-mTOR and its downstream targets, p70S6K and phospho-p70S6K are increased in hippocampus, in agreement with some collected researches [39,40].

In the last decade, mTOR signaling has been extensively reported in AD models, which demonstrated that the aberrant up-regulation of mTOR signaling pathway might be associated with the development of the neurodegenerative process [41,42]. To explore the relationship between Egr1 and mTOR signaling pathway in OVX mice cognitive impairment, OVX mice were treated with the mTOR inhibitor rapamycin. In comparison to vehicle control, the rapamycin treatment negatively regulated Egr1 mRNA and protein expression, in consistent with some other studies [43,44].

Finally, we performed and verified this relationship between Egr1 gene and mTOR signaling pathway in SH-SY5Y cells. The subcellular localization of Egr1 and mTOR was coexisting in SH-SY5Y cells. And there was no alteration in the mTOR and p70S6K mRNA and protein expression after transfection with the si-Egr1 fragment and the Egr1-overexpressing plasmid. Consistent with the above results in hippocampus

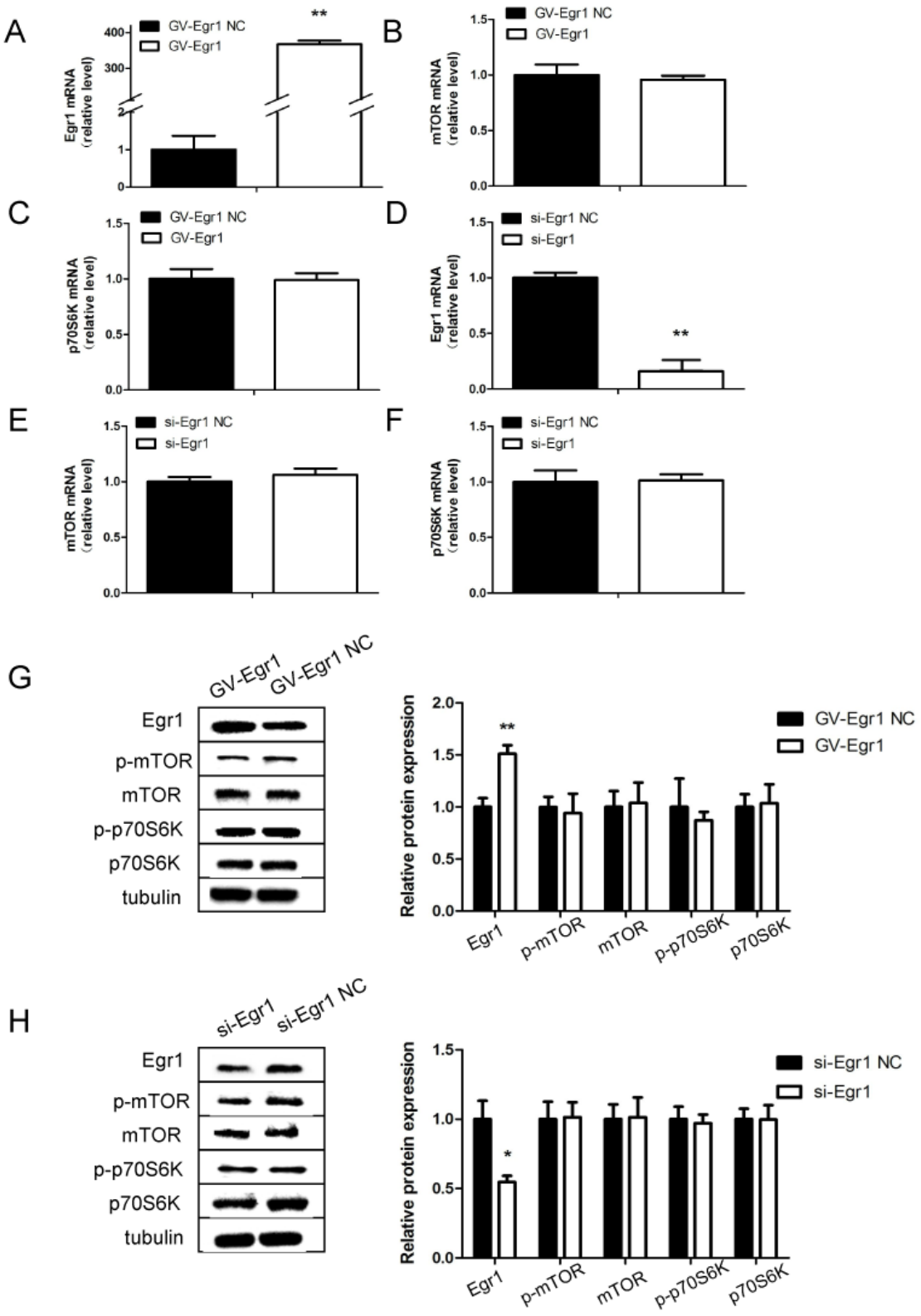

Figure 4: Expression of Egr1, mTOR and p70S6K in SH-SY5Y cells after transfection with Egr1-overexpressing plasmid or si-Egr1 fragment.

Cells were separately transfected with si-Egr1 control (si-Egr1 NC), si-Egr1 fragment (si-Egr1), GV141-Egr1 control (vector), GV141-Egr1 (GV141-Egr1). Egr1 (**A** and **D**), mTOR(**B** and **E**) and p70S6K(**C** and **F**) messenger RNA levels in SH-SY5Y cells after transfection with Egr1-overexpressing plasmid or si-Egr1 fragment. **G, H:** Relative protein levels of Egr1, mTOR and p70S6K after transfection. *P<0.05, **P<0.01.

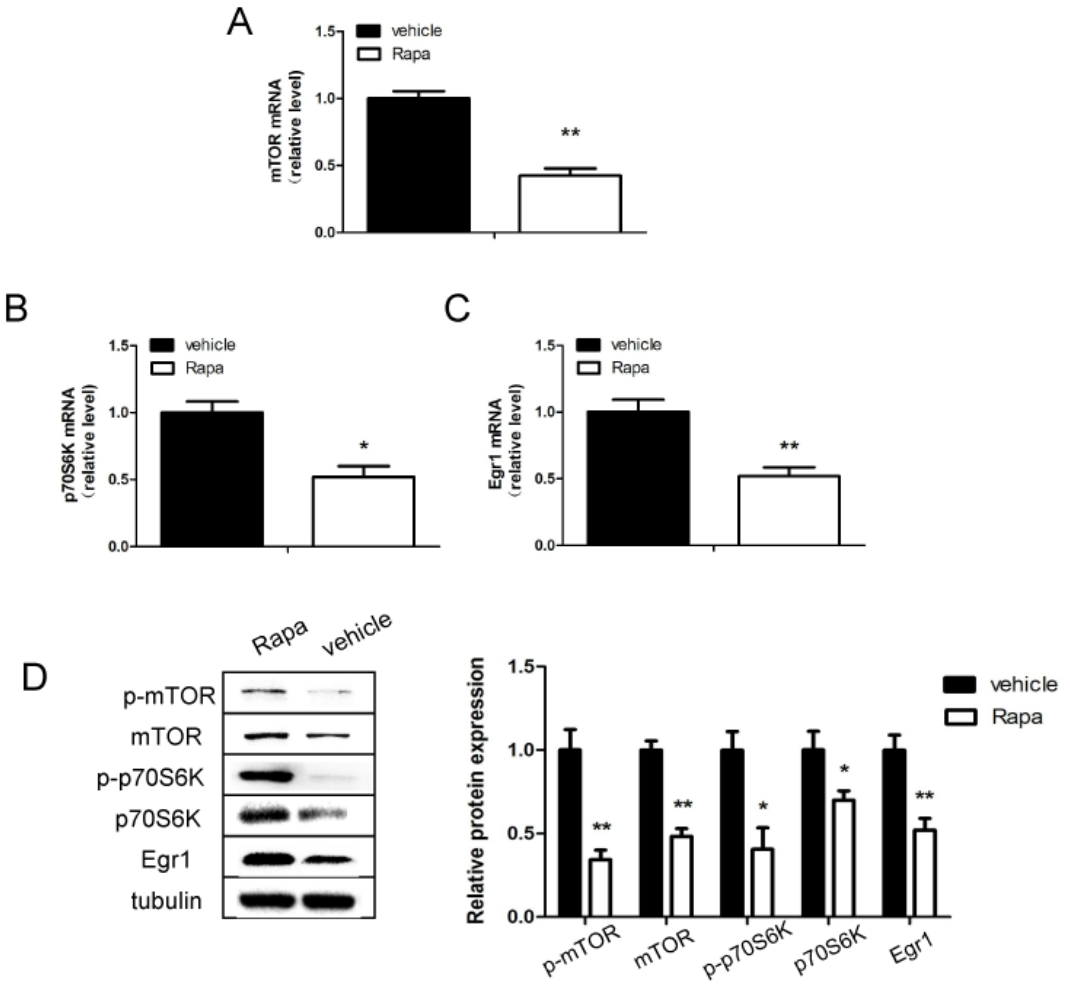

Figure 5: Expression of Egr1, mTOR and p70S6K in SH-SY5Y cells after treatment with rapamycin(4 μM).

mTOR (**A**), p70S6K (**B**) and Egr1 (**C**) messenger RNA levels in SH-SY5Y cells after treated with rapamycin for 48 hours. **D:** Relative protein levels of Egr1, mTOR and p70S6K after treatment with rapamycin for 48 hours. *P<0.05, **P<0.01.

of OVX mice, rapamycin treatment negatively regulated Egr1 mRNA and protein expression, as well as mTOR/p70S6K signaling pathway.

Taken together, our results suggest that the overexpression of Egr1 and mTOR/p70S6K contributes to cognitive decline in OVX mice and Egr1 can be regulated by mTOR/p70S6K signaling *in vivo* and *in vitro* studies. Egr1 maybe a downstream regulator of mTOR/p70S6K signaling pathway in the pathogenesis of cognitive decline. The conclusion was similar to the latest research indicating that Egr1 expression can be regulated by 4EBP1, a classic downstream regulator of mTOR [45]. However, our further studies are needed for a comprehensive understanding of mTOR/p70S6K/ Egr1 and molecular mechanisms of cognitive impairment in OVX model.

So far, there has not effective treatment or conventional drug for cognitive impairment/AD in postmenopausal women. The signaling network of cognition deficiency is complex, with many downstream physiological outputs, and thus the mechanisms underlying its age-related effects have not been fully elucidated. To our knowledge, this study is the first to demonstrate that mTOR/p70S6K/ Egr1 signaling is involved in the development and progress of cognitive dysfunction, which may play an important role in pathogenesis of this disease and be a potential target in a clinical practice of postmenopausal decline.

Conclusion

In summary, our data demonstrated that the over-expression of Egr1 and mTOR/p70S6K contributed to cognitive decline in OVX mice and Egr1 could be regulated by mTOR/p70S6K signaling in vivo and in vitro studies. The results of our current study showed that the mTOR/p70S6K/ Egr1 signaling was involved in the pathogenesis of cognitive dysfunction. Our finding could provide insight into a novel mechanism of the development and progression of postmenopausal decline in clinical practices.

Competing Interests

The authors declare that they have no conflict of interest.

Author's Contributions

SZ: Experiments performance, Data Collection, Manuscript writing, JC: Experiments performance, Data analysis, WZ: Data analysis, JW: Project development, Manuscript editing.

Acknowledgment and Funding

We thank Ling Chen from department of physiology, Nanjing medical university, for kindly providing the technical assistance of electro-

physiological examination. The study was supported by grant from Chinese National Natural Science Foundation (No. 81170540), grant from Jiangsu Province Science and Technology Commission for Natural Science Foundation (No. BK2011846), and grant from Jiangsu Key Laboratory of Neurodegeneration (No SJ11KF06) to Jie Wu.

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