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Research Paper

Estrogen-related receptor alpha is involved in Alzheimer's disease-like pathology

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ABSTRACT

Estrogen-related receptor alpha (ERRα) is a transcriptional factor associated with mitochondrial biogenesis and energy metabolism. However, little is known about the role of ERRα in Alzheimer's disease (AD). Here, we report that in APP/PS1 mice, an animal model of AD, ERRα protein and mRNA were decreased in a region- and age-dependent manner. In HEK293 cells that stably express human full-length β-amyloid precursor protein (APP), overexpression of ERRα inhibited the amyloidogenic processing of APP and consequently reduced $A\beta_{1-40/1-42}$ level. ERRα overexpression also attenuated Tau phosphorylation at selective sites, with the concomitant reduction of glycogen synthase kinase 3β (GSK3β) activity. Interestingly, alterations of APP processing and Tau phosphorylation induced by hydrogen peroxide were reversed by ERRα overexpression in HEK/APP cells. These results indicated that ERRα plays a functional role in AD pathology. By attenuating both amyloidogenesis and Tau phosphorylation, ERRα may serve as a potential therapeutic target for AD.

1. Introduction

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia in the elderly (El Kadmiri et al., 2017). It is estimated that about 24.3 million people had dementia in 2005, with 4.6 million new cases per year, the number of people will double every 20 years, leading to 81.1 million by 2040 (Ferri et al., 2005). The pathological hallmarks of AD are amyloid protein (Aβ) deposition and neurofibrillary tangles (NFTs) (Querfurth and LaFerla, 2010). Although many clinical and experimental studies are ongoing, achieving a cure by simply targeting Aβ or NFTs has proven to be difficult (Mangialasche et al., 2010). Hence, it may be more relevant to identify multifunctional molecules that affect both Aβ generation and NFTs.

Estrogen-related receptor α (ERRα) is a member of orphan nuclear receptor transcription factors, with no known endogenous ligand (Huss et al., 2015). Accumulating evidence has suggested that ERRα plays a central role in regulating gene expression related to mitochondrial biogenesis, oxidative phosphorylation, glycolysis and fatty acid metabolism (Audet-Walsh and Giguere, 2015; Giguere, 2008; Huss et al., 2015; Mootha et al., 2003; Ranhotra, 2015a, 2015b). ERRα also exerts anti-inflammatory activities by regulating gene expression (Yuk et al., 2015). Interestingly, neuroinflammation and oxidative stress are closely associated with AD pathogenesis including Aβ generation and tau-hyperphosphorylation (Jakob-Roetne and Jacobsen, 2009; Querfurth and LaFerla, 2010). It is reported that ERRα has a broad expression in the brain and is regulated by energy status (Cui et al., 2015; Gofflot et al., 2007). On the other hand, reduced energy metabolism in the brain is strongly related to clinical dementia (Jakob-Roetne and Jacobsen, 2009).

We hypothesized that the expression of ERRα in the brain of AD may be altered; and ERRα is functionally associated with AD-like pathogenesis. In this study, we used APP/PS1 mice as an AD model. This model harbors the Swedish mutation (K670N/M671L) of human APP and the mutant presenilin-1 (PS1) gene. Animals develop

Abbreviations: Aβ, amyloid protein; AD, Alzheimer's disease; ADAM10, a disintegrin and metalloproteinase 10; AMPK, AMP-activated protein kinase; APP, β-amyloid precursor protein; APP/PS1, expressing Swedish APP and Presenilin1 delta exon 9 mutations; BACE1, β-amyloid converting enzyme 1; β-CTF, β-COOH-terminal fragment; ERRα, estrogen-related receptor alpha; ERRγ, estrogen-related receptor gamma; HEK/APP, HEK293 cells stably expressing human full-length APP; GSK3β, glycogen synthase kinase 3β; MPP^+, *N*-methyl-4-phenylpyridiniumion; NFTs, neurofibrillary tangles; PPARγ, Peroxisome proliferator-activated receptor γ; shRNA, Short hairpin RNA; WT, wild-type

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amyloidogenesis and Tau-positive neuritis, resembling AD-like pathologies in patients (Bilkei-Gorzo, 2014). In APP/PS1 mice, we assessed the expression of ERRα in the cortex and hippocampus, at 6 mon, 12 mon and 18 mon, respectively. In a cellular model that stably expresses human full-length APP, we assessed the effect of ERRα on Aβ generation and Tau phosphorylation in normal condition and in oxidative stress.

2. Materials and methods

2.1. Animal models

APP/PS1 mice expressing Swedish APP and Presenilin1 delta exon 9 mutations (B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J, # 004462) were purchased from the Model Animal Research Centre of Nanjing University and were maintained in the Experimental Animal Center of Chongqing Medical University. Food and water were available ad libitum. All of the animal procedures conformed to the Ethics Committee of Chongqing Medical University.

2.2. Antibodies and reagents

Antibodies against ERRα (ab76228; 1:1000, for Western blotting), ADAM10 (ab1997; 1:1000), BACE1 (ab2077; 1:1000), total-Tau (ab64193; 1:1000), p-TauT231 (ab151559; 1:1000), p-TauS262 (ab131354; 1:1000), p-TauS396 (ab109390; 1:1000), GSK3β (ab93926; 1:1000) and p-GSK3β-Ser9 (ab75814; 1:1000) were purchased from Abcam (Cambridge, United Kingdom). Anti-ERRα (SC-65718; 1:100, for immunostaining) was from Santa Cruz (California, USA); Antibodies against APP and CTFs (A8717; 1:1000) were from Sigma-Aldrich (St. Louis, MO). Primary antibody against GAPDH or MYC (60003–2-Ig; 1:4000) was from Proteintech (Wuhan, China) and horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies were purchased from Proteintech (Wuhan, China). Immunohistochemistry experiment was performed with Goat hypersensitive two-step detection kit (PV-9003, ZSGB-BIO, Beijing, China).

2.3. Cell culture, plasmid and short hairpin RNA (shRNA) transfection

Human embryonic kidney 293 cell line (HEK293) stably expressing full-length human APP (HEK/APP), was generated as described previously (Hu et al., 2017). The human ESRRA plasmid (HG15911-CM) and control vector pCMV3 (CV013) were purchased from Sino Biological (Beijing, China). Cells were transfected with Lipofectamine™ 2000 Transfection Reagent (11668019, Thermo Fisher Scientific, Inc) and with Opti-MEM Reduced Serum Media (Thermo Fisher Scientific, Inc) according to manufacturer's protocol. The shRNA sequence for human ESRRA (5′-GTGAATGCACTGGTGTCTCAT-3′) was selected from Invitrogen Block-iT RNAi Designer. This sequence was synthesized by Sangon Biotech (Shanghai, China) and designed as follows: 5′-GATC CCC GTGAATGCACTGGTGTCTCAT TTCAAGAGA ATGAGACACCAGT GCATTCAC TTTTT-3′ (sense); and 5′-AGCTAAAAA GTGAATGCACTG GTGTCTCAT TCTCTTGAA ATGAGACACCAGTGCATTCAC GGG-3′ (antisense). These sequences were first annealed and subsequently cloned into pSUPER RNAi empty vector provided by Key Laboratory of Mental Health, Ministry of Health (Peking University). The annealing reaction mixture (50 μl in total) consisted of both of the forward and reverse oligonucleotides, 5 μl 10 × LA Taq buffer Mg^+ free (9153 AM, Takara, Dalian, China) and ddH_2O. The annealing temperature was at 95 °C for 5 min, followed by 70 °C for 10 min before cooling down at room temperature. The pSUPER empty vector was cut in the 50 μl mixture of 2 μl *Hin*dIII (FD0504, Thermo Fisher Scientific, Inc), 2 μl *Bgl*II FastDigest restriction endonuclease (FD0083, Thermo Fisher Scientific, Inc), 5 μl FastDigest buffer, 5 μg pSUPER plasmid and ddH_2O, at 37 °C for 30 min. Digested empty vector was verified by agarose gel electrophoresis and purified using Gel Extraction Kit (D2500-01, Omega, Norcross, GA). Empty vector and target nucleotides were linked in the reaction mixture (15 μl) composed of 1.5 μl 10 × ligase buffer, 1 μl digested empty vector, 5 μl double stranded nucleotides, 1 μl T4 ligase (C301–01-AB, Vazyme, Nanjing, China), and 6.5 μl ddH_2O at 4 °C overnight. The final products were subsequently processed through transformation and colony PCR as described previously (Bergkessel and Guthrie, 2013).

2.4. Western blotting analysis

APP/PS1 mice and wild-type littermate at different age were first euthanized and then the brain was harvested immediately. The cortex and hippocampus on both sides were isolated. Proteins were extracted using RIPA buffer supplemented with protease inhibitors and phosphatase inhibitors (Byotime, Haimen, China) mixture and centrifuged at 12,000 rpm for 15 min at 4 °C. Protein concentrations were measured using a BCA Protein Assay Kit (P0011, Byotime, Haimen, China) and Western blotting were performed as previously described (Hu et al., 2017; Liu et al., 2017). The specific protein bands were visualized using ECL reagent (GE healthcare, UK) and the Fusion FX5 image analysis system (Vilber Lourmat, Marne-la-Vallee, France). Relative protein expression levels were calculated by Quantity One software (Bio-Rad, Hercules, CA) normalized to GAPDH or β-actin.

2.5. Immunohistochemistry

Brain slices from mice (18-month-old) were paraffin-embed and deparaffinized in xylene for 30 min, and were rehydrated in a series concentration of ethanol for a few minutes. After washing with phosphate-buffered saline (PBS, 5 min × 3), brain slices were permeabilized with 0.4% Triton-X 100 for 20 min before antigen retrieval with boiled sodium citrate buffer (10 mM, pH 6.0) for about 15 min, followed by PBS washing and incubation with 3% H_2O_2 (PV-9003, ZSGB-BIO, Beijing, China) for 15 min at 37 °C. Slices were incubated with anti-ERRα antibody overnight at 4 °C. Brain tissues were washed and incubated with reaction enhancement solution (PV-9003, ZSGB-BIO, Beijing, China) for 30 min at 37 °C and with enhanced enzyme-labeled rabbit anti-goat IgG polymer (PV-9003, ZSGB-BIO, Beijing, China) for 30 min at 37 °C. Slices were then treated with 3,3′-diaminobenzidine (DAB, ZSGB-BIO, Beijing, China) for 2 min and PBS. Hematoxylin was used to counterstain nuclei for several minutes. Slices were dehydrated by lithium carbonate for 1 min and incubated with dimethylbenzene for 40 min, then covered and dried overnight. Quantification of immunohistochemistry was calculated by Image-pro Plus 6.0 software (Media Cybernetics, Bethesda, USA) as described previously (Liu et al., 2017).

2.6. RNA extraction and quantitative RT-PCR

Total RNA was extracted from mice brain tissue lysed by using RNAiso plus (Takara, Dalian, China). The cDNA was synthesized by the 5 × HiScript II Select qRT Super Mix II (R233-01-AC, Vazyme, Nanjing, China) according to manufacturer's protocol. mRNA expression levels of ERRα and β-actin were detected by RT-qPCR. The primers for mice ERRα and β-actin were as follows: ERRα, 5′-AGCAAGCCCCGATGGA-3′, and antisense: 5′-GAGAGGCCTGGGATGCTCTT -3′; and β-actin, sense: 5′- ACGGTCAGGTCATCACTATCG -3′, and antisense: 5′-GGCATAGAG GTCTTTACGGATG -3′. Reactions were performed with AceQ qPCR SYBR Green Master Mix (Q111-02, Vazyme, Nanjing, China). The reaction mixture (20 μl total) consisted of 10 μl SYBR, 5.2 μl nuclease-free water, 0.4 μl each primer, and 4 μl diluted cDNA. Reactions were performed using the following steps: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, and the melting curve was run after RT-PCR. The Ct value of each sample was recorded as previously described (Zhu et al., 2016).