ELSEVIER

Neurobiology of Aging 33 (2012) 286–296

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Decreased alternative splicing of estrogen receptor-α mRNA in the Alzheimer's disease brain

Tatjana A. Ishunina[a,b,*], Dick F. Swaab[a]

[a] Netherlands Institute for Neuroscience, An Institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands
[b] Department of Histology, Embryology, Cytology, Kursk State Medical University, Kursk, Russia

Received 18 August 2009; received in revised form 19 February 2010; accepted 16 March 2010

Abstract

In this study we identified 62 estrogen receptor alpha (ERα) mRNA splice variants in different human brain areas of Alzheimer's disease (AD) and control cases and classified them into 12 groups. Forty-eight of these splice forms were identified for the first time. The distribution of alternatively spliced ERα mRNAs was brain area- and case-specific. The dominant negative deletion (del.) 7 isoform appeared to be the major splice variant. The average number of ERα splice forms per brain area was lower in AD cases compared with controls, although the incidence of large deletions with alternative usage of 5′ and 3′ splice sites inside exons was more frequent in AD female cases. Relative transcription levels of del. 7 and del. 2 (missing exon 2) variants were decreased in the temporal cortex of AD patients, whereas the expression of the wild type ERα mRNA did not change. Our data show that alternative splicing of ERα mRNA is diminished in the AD brain and more prominently in AD female cases.

Keywords: Alzheimer's disease; Estrogen receptor α; Human brain; Splice variants

1. Introduction

The higher prevalence of Alzheimer's disease (AD) in women than in men (Andersen et al., 1999) indicates that the decline in estrogen levels around the menopause may be an important factor in the pathogenesis of AD (Genazzani et al., 2007; Naftolin and Malaspina, 2007). Early cohort and case-control studies, indeed, showed that estrogen therapy (ET) in women during menopausal transition was associated with a decreased risk and delayed onset of AD (Craig and Murphy, 2009) if initiated early within the "critical period" when neurons are healthy and can respond to ET (Brinton, 2005). However, no or little benefit of ET on cognitive performance was noticed when given to women with diagnosed AD (Craig and Murphy, 2009; Hogervorst et al., 2009). Because estrogens mediate their effects largely through canonical estrogen receptors (ERs) α and β and because the PvuII and XbaI estrogen receptor alpha (ERα) polymorphisms were linked to both familial and late onset (sporadic) AD (Brandi et al., 1999; Corbo et al., 2006; Ji et al., 2000; Kazama et al., 2004; Lambert et al., 2001; Mattila et al., 2000; Porrello et al., 2006; Yaffe et al., 2002), we investigated whether changes in ERs may occur in the brain areas of AD patients that are involved in cognitive functioning. Nuclear ERα expression was found to be increased in the cholinergic basal forebrain nuclei and in the hypothalamus, but was decreased in the hippocampus (Ishunina et al., 2007b). Brain area-specific changes in ERs in AD patients may have contributed to but did not fully explain the ET pitfalls. Therefore, we hypothesized that the decreased sensitivity of the nervous tissue to ET may be related to changes in the ratio of the canonical and alternatively spliced estrogen receptor-α (ERα) forms. In the present study we investigated this hypothesis and determined the distribution of both canonical and alternatively spliced ERα mRNAs in the human brain by means of the nested polymerase chain reaction (PCR) in frozen samples of different brain areas of Alzheimer's disease (AD) and control cases.

* Corresponding author at: Department of Histology, Kursk State Medical University, Karl Marx Street 3, 305041 Kursk, Russia. Tel.: +789606891979.
E-mail address: T.Ishunina@nin.knaw.nl (T.A. Ishunina).

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2010.03.010

The translated region of the human estrogen receptor alpha gene comprises 8 exons (Greene et al., 1986; Ponglikitmongkol et al., 1988). Exon 1 and a part of exon 2 encode the ligand-independent activation function 1 (AF1) and a coregulatory domain for binding ERα coactivators and corepressors. The remaining portion of exon 2 and exon 3 encode the DNA binding domain for the attachment to estrogen responsive elements within particular genes. Exon 4 codes for the hinge domain, nuclear localization signal, and a part of the ligand binding domain. Exons 5, 6, 7, and 8 encode the ligand binding domain, a portion of the dimerization domain and the ligand-dependent transactivation function 2 (AF2) (reviewed by Perlman et al., 2005 and García Pedrero et al., 2003). In addition to canonical ERα mRNA, these exons may generate a number of splice variants with single or multiple exon skipping, exon duplication, inserts, or partial exon deletions. ERα mRNA splice forms were extensively studied in reproductive organs and particularly in various cancer specimens and cell lines (Taylor et al., 2010). Alternatively spliced ERα mRNAs were also assessed in the human pituitary gland (Chaidarun et al., 1997), mamillary bodies (Ishunina et al., 2005), the hippocampus (Ishunina et al., 2007a) and the prefrontal cortex (Perlman et al., 2005; Weickert et al., 2008). However, no data about the distribution of ERα mRNA isoforms in discrete human brain regions were available thus far.

2. Methods

2.1. Subjects

Three hundred five frozen brain area samples (stored at −80 °C) from 9 AD and 8 control patients (64–76 years old, Table 1, supplementary materials) were obtained at autopsy for the study of ERα splice variants in the human brain in the framework of the Netherlands Brain Bank (NBB). In addition, the presence of ERα splice variants was determined in the hypothalamus of 4 extra cases (Table 2, supplementary materials) and in the hippocampus of 13 extra donors (Table 2, supplementary materials). In all cases the Netherlands Brain Bank received written consent for the brain autopsy and the use of the material and clinical information for research purposes. Medical history of the control cases did not reveal any sign of cognitive disability. The demented patients were clinically assessed and diagnosed as "probable AD" by excluding other possible causes of dementia by history, physical examination, and laboratory tests according to the National Institute of Neurological and Communicative Disorders and Stroke-The Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). Severity of AD was indicated by the Reisberg scale in AD cases (Reisberg et al., 1982). Neuropathological examination of AD patients showed extensive neocortical and hippocampal senile plaques, neurofibrillary tangles, and dystrophic neurites. On the basis of the distribution of neurofibrillary tangles Braak stages were assigned as 0–1 in controls and as 5–6 in AD patients (Braak and Braak, 1991, Tables 1 and 2, supplementary materials).

2.2. RNA isolation and cDNA synthesis

A piece of ~1 cm^3 was excised with a sterile blade (new for each sample) from the frozen brain areas (on average 18 per case) of AD and control patients. A total of 330 RNA samples were then extracted with a Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA yields were around 35–45 μg per sample. RNA integrity was checked by the Agilent 2100 bioanalyzer using the Eukaryote Total RNA Nano assay. There were no differences in the values of the RNA integrity numbers (RIN) between control and AD patients ($p = 0.3$), so the quality of the mRNA did not influence any of the results. RNA integrity numbers numbers were around 5–6, which is acceptable for a PCR study (Fleige and Pfaffl, 2006). Reverse transcription (RT) reactions were carried out using the hexanucleotide mix (Roche Diagnostics, GmbH, Mannheim, Germany). RNA samples were heated to 70 °C for 10 minutes, cooled on ice and mixed with 5× first-strand buffer (Invitrogen), 100 mM DTT, each of the 10 mM dATP, dCTP, dGTP, dTTP (Invitrogen) and 1 μL RNase OUT™ ribonuclease inhibitor (Invitrogen). RT reactions were performed with 200 units of the Superscript II RNase H^- reverse transcriptase (Invitrogen) in a final volume of 20 μL for 1.5 hours at 42 °C.

2.3. Nested RT-PCR

Two microliters of the cDNA were then used as a template to amplify fragments from exon 2 to exon 8. RT-PCR reactions were performed in a final volume of 50 μL containing forward (gaaagattggccagtaccaat) and reverse (taaaatgcagcagggattatct) primers, 0.25 mM dNTPs, SUPER/TAQ buffer, 1.5 mM $MgCl_2$, and 2 units of Taq DNA polymerase (HT Biotechnology, Ltd, Cambridge, UK). The optimal conditions for amplification were as follows: initial denaturation at 94 °C for 2 minutes, 36 cycles corresponding to denaturation at 94 °C for 20 seconds, primers' hybridization at 52 °C for 30 seconds and elongation at 72 °C for 2 minutes; then final elongation at 72 °C for 7 minutes. Two μL of the PCR products were subsequently used for the second round of the nested RT-PCR with a forward (agtatggctatggaatctgc) and a reverse (acttttgcaaggaatgcgatg) primer designed a few bases internally from the position of the first primer pair. No template (sterile water) controls were always negative. The resulting RT-PCR products were loaded on a 1.8% agarose gel stained with ethidium bromide, subjected to electrophoresis at 80 V in TAE buffer and photographed (Sony CCD video camera, Fotodyne Incorporated Foto/Analyst Visionary, Tokyo, Japan) under 300 nm ultraviolet B light (Pharmacia LKB MacroVue, Uppsala, Sweden). The bands were cut out from gels with a sterile blade and the DNA was subsequently isolated according to the Qiaex II agarose gel extraction protocol (Qiagen Benelux B.V., Venlo, The Netherlands). The purified DNA was