Neuroscience 220 (2012) 256–266

EPIGENETIC REGULATION OF *BACE1* IN ALZHEIMER'S DISEASE PATIENTS AND IN TRANSGENIC MICE

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Abstract—In Alzheimer's disease (AD) the complex interplay between environment and genetics has hampered the identification of effective therapeutics. However, epigenetic mechanisms could underlie this complexity. Here, we explored the potential role of epigenetic alterations in AD by investigating gene expression levels and chromatin remodeling in selected AD-related genes. Analysis was performed in the brain of the triple transgenic animal model of AD (3xTg-AD) and in peripheral blood mononuclear cells (PBMCs) from patients diagnosed with AD or Mild Cognitive Impairment (MCI). *BACE1* mRNA levels were increased in aged 3xTg-AD mice as well as in AD PBMCs along with an increase in promoter accessibility and histone H3 acetylation, while the *BACE1* promoter region was less accessible in PBMCs from MCI individuals. *Ncstn* was downregulated in aged 3xTg-AD brains with a condensation of chromatin and *Sirt1* mRNA levels were decreased in these animals despite alterations in histone H3 acetylation. Neither gene was altered in AD PBMCs. The *ADORA2A* gene was not altered in patients or in the 3xTg-AD mice. Overall, our results suggest that chromatin remodeling plays a role in mRNA alterations in AD, prompting for broader and more detailed studies of chromatin and other epigenetic alterations and their potential use as biomarkers in AD.

Key words: Alzheimer's disease, Mild Cognitive Impairment, 3xTg-AD mice, histone acetylation, chromatin remodeling, peripheral blood mononuclear cells.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly. The majority of cases (~99%) have complex etiology due to both environmental and genetic factors, which alone do not seem sufficient for causing disease (Alzheimer's et al., 2011). The role of environmental factors on complex diseases such as cancer or neuropsychiatric disorders can, to some extent, be explained by epigenetic modulation, mediated by DNA methylation, miRNA or chromatin remodeling. Indeed, aberrant DNA methylation, miRNA regulation and histone modification profiles have just begun to be described for neurodegenerative disorders (Hebert et al., 2008; Urdinguio et al., 2009; Chouliaras et al., 2010; Nunez-Iglesias et al., 2010; Long and Lahiri, 2011; Marques et al., 2011).

Epigenetic regulation, involving changes in the micro- and macrostructures of chromatin, differentially alters access of the transcriptional machinery to some genes while leaving access to other genes intact. Chromatin regulation can be accomplished by covalent modification of histones or by the action of ATP-dependent remodeling complexes (Grewal and Jia, 2007). Most genes are subjected to chromatin-mediated transcription regulation and the degree of chromatin effects varies from gene to gene (e.g., gene clusters, tissue-specific genes, housekeeping genes and stress response genes). However, in most cases, acetylation of histones sets the positive epigenetic state for transcriptional activation. Cellular homeostasis is regulated by a fine balance between histone acetylation and deacetylation. Any imbalance in this process leads to aberrant acetylation patterns, with hypoacetylation at loci that should be transcriptionally active and hyperacetylation at genes that should be repressive which together result in disease manifestation (Selvi and Kundu, 2009). Nucleosome dynamics is important because it regulates DNA accessibility, which is a key to proper gene regulation

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Abbreviations: APP, Amyloid Precursor Protein; Aβ, Amyloid-β; AD, Alzheimer's disease; CDR, Clinical Dementia Rating; ChIP, chromatin immunoprecipitation; EDTA, ethylenediaminetetraacetic acid; FAIRE, formaldehyde-assisted isolation of regulatory elements; PBMCs, peripheral blood mononuclear cells; MCI, Mild Cognitive Impairment; M-MSE, Mini Mental-State Evaluation; RT, room temperature; TSS, transcription start site; WT, wild type.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.06.029

Table 1. Epigenetic regulation of the AD-related genes selected for analysis

Gene	Connection to AD	Epigenetic regulation	References
BACE1	Amyloidogenic processing of APP Protein and activity increased in AD brains and CSF	No indication of epigenetic modulation	Roberson et al. (2007) Stozicka et al. (2007)
NCSTN	Critical for γ-secretase stabilization	Age-specific DNA methylation drift in AD brains.	Zhao et al. (2010) Murphy et al. (2003)
	Overexpression increases Aβ production Polymorphisms can increase AD risk		Zhong et al. (2009)
SIRT1	Overexpression reduces γ-secretase in vivo and protects against Aβ toxicity in vitro.	Regulation by miRNA-9 and -181	Chen et al. (2005)
	Lower SIRT1 expression in AD brains		Albani et al. (2009) Donmez et al. (2010) Julien et al. (2009) Schonrock et al. (2012)
ADORA2A	A2AR inhibition prevents Aβ-induced neurotoxicity in vitro and in vivo	Regulation by DNA methylation in human brain	Dall'igna et al. (2003) Canas et al. (2009)

and transcription fidelity. The precise position of nucleosomes around the transcription start site (TSS) has an important influence on the initiation of transcription (Cairns, 2009). Nucleosome loss can occur as a specific response to environmental stresses or signals, leading to transcriptional reprograming (Jiang and Pugh, 2009).

Several studies demonstrated alterations in the global level of histone acetylation in AD or reported protection induced by histone deacetylase inhibitors (HDACi) in disease models. Despite the evidence that histone modifications indeed occur in AD, the pattern of changes is complex and could entail both increases or decreases in histone acetylation at specific loci (Mastroeni et al., 2011). Numerous studies reported transcriptional deregulation of specific genes in AD (Kong et al., 2005; Papassotiropoulos et al., 2006; Wu et al., 2006) but whether this involves post-translational modifications of histones is still largely unknown.

Here, we investigated whether a selected group of four AD-related genes was transcriptionally deregulated since variations in their normal expression are predicted to have a profound impact on the pathology (Table 1). To understand if the chromatin environment in the promoter of the selected genes is altered in AD we also evaluated the epigenetic modulation of the four genes. We performed both analysis in the cortex and hippocampus of the triple transgenic mouse model of AD (3xTg-AD) and in human peripheral blood mononuclear cells (PBMCs) of healthy controls, AD patients, and subjects displaying Mild Cognitive Impairment (MCI), a transitional state between normal aging and AD (Whalley et al., 2006).

EXPERIMENTAL PROCEDURES

Animal brain samples

Colonies of 3xTg-AD mice and wild-type (WT) background strain mice (kind gift from Frank La Ferla, University of California, Irvine, USA) were bred and maintained on 12-h light/dark cycles and provided *ad libitum* access to food and water, in accordance with the European Communities Council Directive (86/609/EEC) on this subject. 6- and 15-month-old WT or 3xTg-AD female mice were sacrificed by cervical dislocation and the brain was removed and divided in the two hemispheres. One half of the cortex and hippocampus were isolated for histone analysis and the other half was used for RNA analysis. All the samples were fast-cooled in liquid N_2 and then kept at −80 °C.

Human PBMCs

A total of 69 subjects (AD patients, MCI individuals and age-matched controls) participated in this study and were recruited at the dementia outpatient clinics, Hospitals of the University, Coimbra, and Hospital Santa Maria, Lisbon. Inclusion for MCI and AD was based on previously reported criteria (APA, 2000; Portet et al., 2006) and in order to implement these criteria, patients were subjected to clinical history, neurological and cognitive examination, laboratorial evaluation and brain imaging (CT-Scan or MRI). Cognitive evaluation included a comprehensive neuropsychological assessment using a normalized battery for the Portuguese population and the Mini Mental-State Evaluation (MMSE) (Folstein et al., 1975). Furthermore, severity of disease was classified according to the Clinical Dementia Rating Scale (CDR) (Morris, 1993) where a rating of zero indicates no cognitive abnormality, score 0.5 questionable or suspected deterioration and scores of 1, 2 or 3, respectively mild, moderate and severe dementia. Control subjects did not present evidence of cognitive deterioration or cognitive complaints, had a MMSE above cut-off for the Portuguese population and their value in the CDR was zero. The exclusion criteria for all groups were the presence of other neurological, psychiatric or medical pathologies that could cause cognitive impairment, or a history of alcohol or drug abuse. The protocol investigation was approved by the Ethics Committees of both Portuguese clinical institutions and all participants, or respective caregivers, signed an informed consent before any procedure. Demographic and clinical characteristics of the participants are shown in Table 2.

Peripheral blood was withdrawn into EDTA-vacuum tubes and PBMCs were extracted using Ficoll-Paque (GE Healthcare, Waukesha, WI, USA). PBMCs pellets were stored at −80 °C until use.

Reverse transcription-polymerase chain reaction

RNA was extracted by means of the Trizol® reagent. cDNAs were then obtained by reverse transcription reaction using the iScript Supermix in a My Cycler equipment (Bio-Rad Laboratories Headquarters, Hercules, CA, USA) and were measured by real-time