Neurobiology of Aging 34 (2013) 2081–2090

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Disruption of neocortical histone H3 homeostasis by soluble Aβ: implications for Alzheimer's disease

Christina Unger Lithner [b], Pascale N. Lacor [c], Wei-Qin Zhao [c], Tamanna Mustafiz [b], William L. Klein [c], J. David Sweatt [a], Caterina M. Hernandez [a,*]

[a] McKnight Brain Institute, Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA
[b] Alzheimer Neurobiology Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden
[c] Department of Neurobiology, O.T. Hogan Biological Sciences, Northwestern University, Evanston, IL, USA

ARTICLE INFO

Article history:
Received 22 August 2012
Accepted 28 December 2012
Available online 9 April 2013

Keywords:
Alzheimer's disease
Epigenetics
Histone H3
Histone acetylation
Histone methylation
Amyloid-β peptide/protein
Soluble Aβ/Aβ-derived diffusible ligand (ADDL)
neocortex
Drebrin
Tg2576 transgenic mouse model
Dendritic spine

ABSTRACT

Amyloid-β peptide (Aβ) fragment misfolding may play a crucial role in the progression of Alzheimer's disease (AD) pathophysiology as well as epigenetic mechanisms at the DNA and histone level. We hypothesized that histone H3 homeostasis is disrupted in association with the appearance of soluble Aβ at an early stage in AD progression. We identified, localized, and compared histone H3 modifications in multiple model systems (neural-like SH-SY5Y, primary neurons, Tg2576 mice, and AD neocortex), and narrowed our focus to investigate 3 key motifs associated with regulating transcriptional activation and inhibition: acetylated lysine 14, phosphorylated serine 10 and dimethylated lysine 9. Our results in vitro and in vivo indicate that multimeric soluble Aβ may be a potent signaling molecule indirectly modulating the transcriptional activity of DNA by modulating histone H3 homeostasis. These findings reveal potential loci of transcriptional disruption relevant to AD. Identifying genes that undergo significant epigenetic alterations in response to Aβ could aid in the understanding of the pathogenesis of AD, as well as suggesting possible new treatment strategies.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by memory loss and cognitive decline, and is the most common form of dementia worldwide. The hallmark pathological features of AD involve misfolding and aggregation of 2 proteins including the extracellular accumulation of amyloid-β peptide (Aβ) in plaques and aggregation of hyper-phosphorylated tau in neurofibrillary tangles. Although genetic and biochemical studies suggest a cardinal role for Aβ and its expression precedes dementia by many years (Davis et al., 1999), the mechanism by which Aβ induces neurodegeneration is unclear. Only a minimal percentage of all AD cases are early onset and arise through autosomal inheritance of 1 of several causative genetic mutations, including the genetic precursor to Aβ, the amyloid precursor protein (APP) gene (Selkoe, 2001). The majority of AD cases are sporadic and are not attributed to specific genetic mutations.

The etiology and neuropathological progression of sporadic AD is associated with multiple environmental events and mechanisms. During the progression of AD pathophysiology, the interplay between genetic and environmental factors may play a crucial role. Gene expression can be modulated without changes to DNA sequence, by epigenetic mechanisms such as histone modifications, binding of non-histone proteins and DNA methylation. Although epigenetic changes are often heritable, they may also be induced spontaneously and in response to environmental factors, and such modifications may critically contribute to the progression of AD pathophysiology. (Bannister and Kouzarides, 2011; Waddington, 1957).

It is known that epigenetic modifications by DNA methylation are altered in the promoter region of neuronal genes in AD (Lee and Ryu, 2010), and histone methylation and phosphorylation are increased in AD brain as well as in response to numerous signaling events (Lee and Ryu, 2010; Ogawa et al., 2003; Sweatt, 2010). In addition, AD and AD mouse models (associated with aberrant APP processing) demonstrate altered gene transcription and histone

* Corresponding author at: Department of Pharmacology and Toxicology, Georgia Regents University, 1120 15th Street, CB 3545, Augusta, GA, USA. Tel.: +17067219514; fax: +17067212347.
E-mail address: caterina.hernandez@gmail.com (C.M. Hernandez).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.12.028

acetylation (Chouliaras et al., 2010; Kilgore et al., 2010; Robakis 2003; Walker et al., 2012). Using both in vitro and in vivo model systems, we sought to identify and localize changes in epigenetic marks at the histone level associated with Aβ secretion and exposure using SH-SY5Y^{APPSWE} cells, primary cortico/hippocampal neurons, Tg2576 (a human APP^{SWE} transgenic mouse model), and AD occipital cortex. During the early stages of AD, even in prodromal AD, it is a challenge to detect Aβ in brain and serum and to identify the prevalent structure (i.e., monomeric, dimeric, trimeric, oligomeric, etc.) (Bao et al., 2012; Fukumoto et al., 2010; Gong et al., 2003; Klyubin et al., 2008; Lesne et al., 2006; McDonald et al., 2012; Selkoe, 2008). A significant Aβ load in brain, measured biochemically or by PET imaging, is highly correlated with progression from mild cognitive impairment to AD (Brys et al., 2009; Forsberg et al., 2008; Hansson et al., 2006; Mattsson et al., 2009; Nordberg, 2011; Okello et al., 2009;Visser et al., 2009; Waragai et al., 2009; Wolk et al., 2009). PET amyloid imaging (using Pittsburgh compound B, a thioflavin T fluorescent analog) also indicates that amyloid deposition in the brain occurs in non-demented elderly individuals and before the onset of cognitive symptoms in AD patients (Chetelat et al., 2011; Pike et al., 2007; Villemagne et al., 2008). Moreover, increased cortical PIB is associated with discrete episodic memory impairments in healthy (i.e. non-demented) elderly subjects (Pike et al., 2007). In addition, many AD mouse models demonstrate the same trends in amyloid load (Duyckaerts et al., 2008; Morrissette et al., 2009). Many studies demonstrate that soluble Aβ (Aβ$_{sol}$) oligomers cause early-onset structural and functional changes to neurons and may be the trigger for cognitive dysfunction and decline observed in AD (Cleary et al., 2005; Hu et al., 2008; Jin et al., 2011; Klyubin et al., 2008; Lacor et al., 2004; Lacor et al., 2007; Lambert et al., 1998; Lesne et al., 2006; Mucke et al., 2000; Renner et al., 2010; Selkoe 2002; Selkoe 2008; Shankar et al., 2007; Walsh et al., 2002; Zempel et al., 2010).

Our hypothesis is that Aβ$_{sol}$ induces disruption of histone homeostasis early during the progression of AD and, in turn, is involved in the progressive cognitive decline observed in AD patients. Work from our laboratory and others' has established that epigenetic mechanisms at the histone level, particularly histone H3 (Chwang et al., 2006; Levenson et al., 2004), are important for learning and memory function (Day and Sweatt, 2011b). The objective of the present studies was to determine whether epigenetic changes at the histone level were associated with Aβ$_{sol}$ oligomers, and furthermore, whether changes are detectable before or after the onset of plaques and memory dysfunction in an AD murine model and human AD brain. We narrowed our focus to investigate total histone H3 (TH3) and 3 key modifications associated with regulating transcriptional activation and inhibition: acetylated lysine 14 (AcH3), phosphorylated serine (S) 10 (PH3), and dimethylated lysine 9 (2MeH3). Our results indicate that Aβ$_{sol}$ oligomers may be a potent signaling molecules indirectly modulating transcriptional activity by acetylating and methylating H3 lysine residues.

2. Methods

2.1. Chemicals, reagents, and antibodies

All chemicals and reagents were purchased from Sigma-Aldrich. Epitope-specific antibodies (Millipore) were used to target histone H3 and motifs: total histone H3 (TH3), acetyl-K14-H3 (AcH3), phospho-S10-H3 (PH3), and dimethyl-K9-H3 (2MeH3). Others used were as follows: anti-: NU-1 or 2 (strong preferential binding for Aβ oligomers over monomers) (Lambert et al., 2007), drebrin (Stressgen), neuronal nuclear antigen (NeuN, Millipore), and AlexaFluor (Invitrogen), biotin (Jackson ImmunoResearch), and horseradish peroxidase (HRP; Vector Labs)–conjugated secondary antibodies.

2.2. Aβ-derived diffusible ligands preparation

Aβ derived diffusible ligands (ADDLs), also referred to as Aβ$_{sol}$ oligomers (Klein, 2002), were prepared as previously described (Klein, 2002; Lacor et al., 2004; Lacor et al., 2007; Lambert et al., 2007).

2.3. Cell culture and treatment

For all cell culture experiments, fresh media changes were conducted before all treatments. Cells were rinsed before harvesting and were stored at −80 °C.

2.3.1. SH-SY5Y cells

Stably transfected SH-SY5Y, a human neuroblastoma cell line, expressing human APP with the Swedish K670N/M671L double mutation (APP^{SWE}) or an empty (pcDNA 3.1) vector containing cytomegalovirus promoter (vector) as a control, were a kind gift from Dr E Benedikz, Karolinska Institutet, Sweden (Zheng et al., 2009). Cells are referred to as vector and SH-SY5Y^{APPSWE} for the remainder of this article and were cultured and maintained under sterile conditions in RPMI-1640 (Invitrogen) supplemented with fetal bovine serum and gentamicin (Life Technologies). For SH-SY5Y treatments, LY-374973, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-sphenylglycine t-butyl ester (DAPT), a γ-secretase inhibitor, was used to reduce secreted Aβ into culture media. Cells were treated with 100 μmol/L DAPT prepared in DMSO (vehicle) and harvested at 16 hours. Secreted Aβ was measured in media using a MULTI-SPOT human (6E10) Abeta Triplex Assay kit (Mesoscale Discovery), recognizing Aβ monomers and oligomers, according to package instructions. Sodium butyrate (NaB), a histone deacetylase inhibitor (HDACi), was used to prevent acetyl group removal from histone lysine residues. Cells were harvested after treated with 400 μmol/L NaB prepared in ultra-pure H_2O (vehicle) for 4 hours. Three culture experiments were performed with each treatment in duplicate.

2.3.2. Primary cortico/hippocampal neurons

Under aseptic conditions at P1 and E18, respectively, cortical and hippocampal tissues were isolated from Sprague-Dawley rats (Charles River) and trypsinized. Cells (density = 104 cells/cm^2) were maintained on poly-L-lysine–coated coverslips for 14 to 21 days in vitro in Neurobasal media (Invitrogen) supplemented with B27, L-glutamine, and antibiotics. Three culture experiments were performed with each treatment in duplicate. Primary neuronal culture treatments were as follows. For the immunocytochemical (ICC) and biochemical (BC) studies, neurons were pre-incubated for 3 hours in 20 mmol/L HEPES (containing [mmol/L]: 125 NaCl, 5 KCl, 1 Na_2HPO4, 1 $MgSO_4$, 1 $CaCl_2$, 5.5 D-glucose) before ADDLs (100–200 nmol/L, ICC: 30 min or BC: 1 or 6 hours). For dendritic spine analyses, neurons were pretreated for 30 minutes with 25 μmol/L Garcinol (Biomol), a potent histone acetyltransferase inhibitor derived from *Garcinia indica* fruit rind (Balasubramanyam et al., 2004; Mantelingu et al., 2007), before ADDLs (500 nmol/L, 24 hours).

2.4. Animal subjects

For primary neuronal cultures, we used embryos and pups from timed pregnant Sprague-Dawley rats purchased from Charles River. Three-month-old (MO) male transgenic mice (Tg2576) overexpressing human amyloid precursor protein carrying the Swedish K670N/M671L double mutation (Hsiao et al., 1996) and non-transgenic littermates (wild-type, WT) were purchased from Taconic (Hudson, NY); the background strain is an F1 hybrid of SJL and C57Bl/6Tac. Genotypes were determined by PCR of genomic