Published in final edited form as:

Neurobiol Aging. 2015 December ; 36(12): 3121–3129. doi:10.1016/j.neurobiolaging.2015.08.017.

Aberrant intracellular localization of H3k4me3 demonstrates an early epigenetic phenomenon in Alzheimer's disease

Diego Mastroeni^{a,b,c}, Elaine Delvaux^{a,b}, Jennifer Nolz^{a,b}, Yuyan Tan^d, Andrew Grover^b, Salvatore Oddo^{e,f}, and Paul D. Coleman^{a,b,*}

^aASU-Banner Neurodegenerative Disease Research Center, Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, AZ, USA ^bL.J. Roberts Alzheimer's Disease Center, Banner Sun Health Research Institute, Sun City, AZ, USA ^cDepartment of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNS), Faculty of Health, Medicine and Life Sciences, European Graduate School of Neuroscience (EURON), Maastricht University Medical Centre, Maastricht, The Netherlands ^dDepartment of Neurology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China ^eOddo Laboratory—Neurobiology of Aging and Dementia, Banner Sun Health Research Institute, Sun City, AZ, USA ^fDepartment of Basic Medical Sciences, University of Arizona College of Medicine-Phoenix, Phoenix, AZ, USA

Abstract

We have previously reported in Alzheimer's disease (AD) the mislocalization of epigenetic molecules between the cell nucleus and the cytoplasm. We have extended our finding to include the aberrant localization of histone 3 trimethylation on lysine 4 (H3k4me3), an epigenetic mark associated with actively transcribing genes as well as those poised for transcription. These findings raise the question of where the ectopic localization of H3k4me3 fits within the cascade of cell biological events in the progression of AD. We, therefore, examined the expression and intracellular location of H3k4me3 as a function of Braak stage and also in relation to a series of tau markers that are indicative of disease state. Both lines of evidence showed that ectopic localization of H3k4me3 is early in the course of disease. Because of the known role of H3k4me3 in the expression of synaptic genes, our data suggest an epigenetic role in synaptic deficits early in the course of AD.

Keywords

Alzheimer's disease; Epigenetics; H3k4me3; Localization

*Corresponding author at: ASU-Banner Neurodegenerative Disease Research Center, Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, AZ, USA. paul.coleman@bannerhealth.com (P.D. Coleman).

Disclosure statement

The authors have no conflicts of interest to disclose.

1. Introduction

Alzheimer's disease (AD) is a multifactorial disorder, encompassing multiple pathogenic pathways affecting multiple brain regions. The classic neuropathological lesions defining this disease are plaques and neurofibrillary tangles (NFT), reviewed in Perl (2010). Braak and Braak have defined AD progression from early to final stage disease on the basis of the progressive presence of NFT in successive brain regions, with stage I being the earliest, affecting limbic or brain stem regions, and widespread neocortical stage VI, the latest disease stage (Braak and Braak, 1991; Braak and Del Tredici, 2011; Braak et al., 2011). The chief component of NFT is tau (reviewed in Wang et al., 2013) which undergoes successive phosphorylation, conformation and other changes at selected sites as disease progresses (Augustinack et al., 2002). Davies et al. have developed antibodies to a number of phosphorylated and conformational dependent epitopes of tau, some of which have been shown to precede the formation of NFT (Davies, 2000; Jicha et al., 1997; Weaver et al., 2000). PG5 recognizes phospho-serine 409, and MC1 (a conformation-dependent antibody) recognizes phosphorylation on serine 312–322. Both have been shown to be early markers of disease (Davies, 2000; Jicha et al., 1997; Weaver et al., 2000). Here, we show that the cytoplasmic localization of the epigenetic mark, histone 3 trimethylation on lysine 4 (H3k4me3) is associated with and even precedes the early phosphorylation markers MC1 and PG5.

Post-translational modifications on histone 3 have been shown to be a critical intermediate in shaping gene expression during normal aging and disease (Bannister and Kouzarides, 2002; Berger, 2002; Fraga and Esteller, 2007; Gilbert et al., 2007; Jaenisch and Bird, 2003). Presence of H3k4me3 in the nucleus has been shown to facilitate the efficiency of the post-initiation processes of active transcription (Sims et al., 2007) and is found globally at >90% of RNA polymerase II binding sites (Barski et al., 2007). Furthermore, histone methylation has been shown to directly affect chromatin structure and consequently gene expression (Bannister and Kouzarides, 2002). H3k4me3 is of interest in studies of neurodegenerative disease, including Alzheimer's, because of its relation to gene expression, its influence on learning and memory (Gupta et al., 2010), and synaptic transmission (Cheung et al., 2010).

Examination of human AD and control brain by immunohistochemistry and Western blot revealed cytoplasmic accumulation of H3k4me3. These findings were highly correlated with increasing Braak stage, as well as in relation to antibodies indicating presumptive successive stages of tau post-translational processing. Early Braak stages from nondemented (ND) samples revealed cytoplasmic H3k4me3 more often than any of the tau markers examined. Successive Braak stages were also accompanied by decreased immunoreactivity (IR) for H3k4me3 in the nuclear compartment. These data indicate cytoplasmic accumulation of H3k4me3 as an early event in AD cell pathology. We also replicate the nuclear loss but not the cytoplasmic accumulation of H3k4me3 in a mouse model of AD.

2. Methods

2.1. Subjects, brain samples, and cells

Samples of human brain, containing hippocampus, midbrain, and middle temporal gyrus were secured from AD, ND, and Parkinson's (PD) brains obtained at autopsy at the Banner Sun Health Research Institute Tissue Bank. Cognitive status of all cases was evaluated antemortem by board-certified neurologists, and final diagnosis was made postmortem by a board-certified neuropathologist, resulting in a consensus diagnosis using standard National Institutes of Health AD Center criteria for AD or neurologically normal, ND elderly control (Beach et al., 2008). The AD and ND groups were well-matched for age, gender, and postmortem interval (PMI), see Table 1 for individual details.

ND cases: 84 ± 7.9 years: Braak 0 (no pathology): $n = 4$, Braak II: $n = 4$, Braak III: $n = 5$, Braak IV: $n = 6$, 19 total control cases. Gender: 11 females and 8 males. PMI: 2.86 hours $\pm$ 76 minutes.

AD cases: 85 ± 8.9 years: Braak IV: $n = 10$, Braak VI: $n = 8$, 18 total AD cases. Gender: 9 females and 9 males. PMI: 3.14 hours $\pm$ 1 hour.

PD cases: 78.5 ± 5.6 years: Braak 0–I (no AD pathology): $n = 4$. Gender: 4 females. PMI: 3.3 hours $\pm$ 91 minutes.

Animal studies: male and female 3xTg-AD mice and their respective nontransgenic littermates were anesthetized and perfused with 4% buffered paraformaldehyde. Brains were sectioned at 40 μ m to contain hippocampus. For further detail on genetic background and brain extraction and fixation method refer to Oddo et al. (2003a), (2003b). Transgenic mice ages were 8 months ($n = 7$), 12 months ($n = 3$), 15 months ($n = 4$), and 19 months ($n = 7$).

2.2. Immunohistochemistry

DAB method: 40- μ m free-floating sections were washed in phosphate-buffered saline Triton X-100, blocked in 1% hydrogen peroxide followed by 1 hour incubation in 3% bovine serum albumin (BSA), then incubated at 4 °C overnight in primary antibody solutions containing 0.25% BSA. Sources and dilutions for antibodies are given in Table 2. After incubation, the sections were washed, incubated in biotinylated, species-specific secondary antibodies (Vector) for 2 hours at room temperature, washed 3 $\times$ in phosphate-buffered saline Triton X-100, and incubated in avidin-biotin complex (Pierce) for 30 minutes. After incubation with avidin-biotin complex, sections were washed 2 $\times$ in 50-mM Tris buffer and immersed in DAB solution (500- μ L 5 mg/mL DAB, 2-mL saturated nickel, 10- μ L 1% H₂O₂, QS to 50 mL with 50-mM Tris buffer) for no longer than 10 minutes, followed by 2 quick rinses in 50-mM Tris to stop the reaction. AD and ND sections were immunoreacted simultaneously using netwells in well-less plates. Sections were mounted with Permount (Pierce). Same methods as previously mentioned were used with 8 μ m paraffin-embedded sections with the exception of the use of xylene for deparaffinization.

Fluorescence microscopy, paraffin embedded or 40- μ m free-floating sections were washed and blocked as mentioned previously. Sections were incubated at 4 °C overnight in primary

antibody solutions containing 0.25% BSA, washed again, and incubated in species-specific, fluorophore-conjugated secondary antibodies (Molecular Probes) for 2 hours. After a final wash, the sections were mounted, taken through Sudan Black to reduce autofluorescence, and coverslipped with Vectashield (Vector). Deletion of primary antibody or incubation with preimmune serum resulted in abolition of specific IR in all cases (data not shown).

Immunostained tissue sections were examined on Olympus IX51 and Olympus IX70 microscopes equipped with epifluorescence illumination or with confocal laser scanning using argon and krypton lasers (Olympus IX70). The findings were documented photographically with an Olympus DP-71 color digital camera or, for confocal microscopy, by Fluoview software (Olympus). After photographic imaging, cellular localization was quantified using ImageJ software.

Quantification of nuclear versus cytoplasmic: bright-field intensity analysis was performed using ImageJ software. Intensity measurements were corrected for background differences by dividing the measured intensities with the average intensity of a background region. Neurons were evaluated by delineating the nucleus (dashed circle) from cytoplasm (solid black line), see Fig. 1, of each cell and measuring the mean intensity of each area (i.e., nuclear and cytoplasmic).

2.3. Nuclear and cytoplasmic preparations

Nuclear extract kits (Active Motif) were used for nuclear and/or cytoplasmic fractionation. Briefly, fresh brain tissue was chopped into 1-cm pieces and resuspended in 3-mL hypotonic buffer. Tissue slurry was transferred to a Dounce homogenizer (large pestle clearance 0.12 mm for initial sample reduction) and mechanically dissociated on ice (approximately 20 strokes). Samples were incubated on ice for 15 m and centrifuged at 850g for 10 minutes at 4 °C. Supernatant was removed and put aside on ice (cytosolic fraction). Nuclear pellet was resuspended in 500-μL 1× stock hypotonic solution (previously mentioned), and incubated on ice for 15 minutes. Twenty-five microliter of Tween-20 was added and vortexed at maximum speed for 10 seconds. Samples were then centrifuged at 14,000g for 30 seconds at 4 °C to pellet nuclei. Supplementary Fig. 1 shows the reliability of separating cytoplasmic and nuclear fractions. There does, however, seem to be minuscule amounts of cytoplasmic and nuclear leakage in both preparations, but the Western blot shows only minimal reactivity.

2.4. Western blot

Nuclear and cytosolic preparations isolated from mid temporal gyrus were lysed in a solution containing 20-mM Tris, pH 7.5; 0.5% Nonidet (Sigma), 1-mM EDTA (Sigma), 0.1-M NaCl (Sigma), 1-mM PMSF (Sigma), Sigma protease inhibitors 1, 2, and complete protease inhibitor cocktail (Roche). Protein concentrations were determined by BCA assay (Pierce). Twenty micrograms of sample protein was combined with Laemmli sample buffer for separation by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by transfer to PVDF membrane (Bio-Rad). Membranes were blocked using 5% BSA and probed with primary antibodies (Table 1). Membranes were washed, incubated with secondary antibody, washed again, reacted with chemiluminescence substrate (Pierce),

imaged on an Alpha Ease detection system, and analyzed using AlphaEaseFC software (Alpha Innotech).

2.5. Enzyme-linked immunosorbent assay

To determine total H3k4me3 levels, PathScan Sandwich enzyme-linked immunosorbent assay (ELISA) (Cell Signaling) was used. Ten AD and 10 ND age- and/or sex- and/or PMI-matched frozen mid temporal gyrus. Protein isolation and quantification was executed identical to Section 2.4. A total of 20 µg of sample protein was diluted 1:1 with sample diluent and loaded into each well. The plate was incubated for 2 hours at 37 °C, followed by washing steps and secondary antibody. Samples were read on the Wallac 1420 Victor2 at 450 nm absorbance.

2.6. Statistical analyses

Significance was determined using a 2-tailed student *t* test and declared significant at a *p*-value < 0.05. Correlation analysis was determined by Pearson product-moment correlation coefficient analysis. Significance was determined at the 0.05 level.

3. Results

3.1. Cytoplasmic localization of H3k4me3 in CA1 of hippocampus in control and AD brains as a function of Braak stage

In clean, no pathology controls, H3k4me3 IR in CA1 of hippocampus revealed no obvious cytoplasmic accumulation but showed strong IR within the nuclear compartment (Fig. 1A). Braak II ND cases (the earliest Braak stage that showed consistent tangle pathology) show occasional diffuse cytoplasmic H3k4me3 IR (Fig. 1B), followed by successive increases in cytoplasmic H3k4me3 in Braak III and IV ND cases, respectively (Fig. 2). Regression analyses revealed significant positive correlation between cytoplasmic H3k4me3 IR and increasing Braak stage in controls ($r_p = 0.97$, $p = 0.001$) and AD cases ($r_p = 0.99$, $p = 0.0001$) (Fig. 2). However, comparing equivalent Braak stages in AD and controls (IV, the lowest Braak stage most often associated with an AD diagnosis in our Brain Bank) showed a 30% increase in cytoplasmic IR in AD, although both control and AD cases were equal in terms of pathology (Fig. 2). We believe this discrepancy between the Braak IV AD and Braak IV ND cases may be due to additional differences between AD and ND neurons.

3.2. H3k4me3 localization in Braak IV AD is related to position of nucleus within cells

Cytoplasmic H3k4me3 IR in neurons containing a displaced nucleus was most often observed as elongated inclusions extending into the apical dendrites (Fig. 1C). Neurons with a central nucleus showed only modest cytoplasmic IR (Fig. 1C, arrowhead). In addition to the cytoplasmic accumulation in neurons, occasional IR could be observed in neurites in AD cases (see Fig. 1C for more details). Neurons which showed a central nucleus showed significantly less ($p = 0.0025$) nuclear IR compared to neurons containing a displaced nucleus (Fig. 1C arrowhead, also see 1C inset). These data show that H3k4me3 localization may be associated with intracellular nuclear position, presumably related to displacement of the nucleus by NFT.

3.3. Quantitative analysis of H3k4me3 localization

To quantify the immunohistological observations, intensity of IR, Western blots, and ELISAs were performed on the earliest and most consistent, pathologically confirmed Braak stage in AD (IV), and the first signs of consistent tau pathology in controls (Braak II). Quantification of fluorescence intensity in cytoplasmic and nuclear compartments confirmed greater nuclear intensity in ND cases and greater cytoplasmic intensity in AD cases (Fig. 1D). Quantification of signal intensity of cytoplasmic and nuclear IR by Western blot showed that AD cytoplasmic preparations were significantly more reactive for H3k4me3 ($p = 0.02$) compared to ND subjects. This increased cytoplasmic IR in AD was accompanied by decreased nuclear IR for H3k4me3 in AD ($p = 0.0001$), with ND expressing 3 times the amount of nuclear H3k4me3 than AD nuclear preparations (Fig. 1E). In contrast, whole-cell protein extracts analyzed by ELISA, showed no significant difference between Braak IV AD and Braak II control cases (Fig. 1F). These data point out the importance of immunohistochemical analysis in combination with Western blot and ELISA data which may not, under usual circumstances, reveal potentially crucial differences in intracellular localization. Ectopic localization of h3k4me3 was not observed in the affected nigral neurons in PD brains but was in AD nigral neurons, indicating that these findings are specific to AD rather than a generalized neurodegenerative phenomenon (Supplementary Fig. 2).

3.4. Cytoplasmic localization of H3k4me3 precedes early tau markers

To determine where the cytoplasmic accumulation of H3k4me3 fits in the cascade of cell biological events in the progression of AD, we examined Braak 0 and Braak II controls versus Braak IV AD cases. We chose Braak 0 controls to determine whether this is an aging phenomenon and Braak II controls because it was the earliest Braak stage that showed tangle pathology and Braak IV AD cases because it was the lowest Braak stage most often associated with an AD diagnosis in our Brain Bank. In CA1 hippocampus Braak 0 ND cases, however, showed no IR for either early tau markers PG5 or MC1, although Braak II ND cases did show occasional cytoplasmic IR for H3k4me3 (<5%) of cells analyzed (see Fig. 3 and Fig. 1D). These results show that H3k4me3 cytoplasmic IR not only is found in association with PG5 and MC1 IR but that it also precedes these early tau marks in Braak II ND cases. Braak IV AD cases were more or less used as positive controls, which showed strong cytoplasmic IR for PG5, MC1, and H3k4me3 (Fig. 3).

3.5. Association between H3k4me3 and phosphorylated tau

To determine the relationship of cytoplasmic accumulation of H3k4me3 with selected markers of tau phosphorylation, we used double-label immunohistochemistry with confocal microscopy in Braak IV AD cases and in high-pathology controls (Braak IV) (Fig. 4). We selected high pathology controls for this study to compare samples with similar pathological states. These data show that when CA1 neurons ectopically express H3k4me3 in Braak IV AD or Braak IV ND cases, the most significant association is with early tau markers PG5 ($r_p = 0.92$, $p = 0.001$) and MC1 ($r_p = 0.9$, $p = 0.003$). Further analysis also shows that although CP13 and PHF-1-positive neurons do not colocalize with cytoplasmic H3k4me3 as often as PG5 or MC1, both cytoplasmic H3k4me3 and CP13 and/or PHF-1 IR are found often in the

same cells. These data indicate that there is a high probability that cytoplasmic H3k4me3 will be present when any of the 4 tau markers tested are present.

3.6. 3xTg-AD mouse model of AD replicates select aspects of human findings

Representative photomicrographs of the 3xTg-AD mouse model of AD shows qualitative similarities in the nuclear loss of H3k4me3 IR to that seen in human AD samples in an age- or disease-dependent manner (Fig. 5). Further analysis within the subregions of the hippocampus reveals regional effects that differ from those seen in human AD. The 3xTg-AD mice show the most significant loss of nuclear H3k4me3 in the CA3 (Fig. 5, arrow and Fig. 5E) rather than CA1, as seen in humans. As age and disease pathogenesis increases the distribution of IR shifts from CA3 to CA2 followed by only modest changes in the CA1 by 19 months (Fig. 5E). Although nuclear loss was evident by 19 months, cytoplasmic accumulation was absent in all regions analyzed (Fig. 5).

4. Discussion

The normal localization of H3k4me3 and other epigenetic molecules is typically within the nuclear compartment (Espada and Esteller, 2007), where they play a pivotal role in chromatin structure (Sims et al., 2007), and the regulation of gene expression (Jaenisch and Bird, 2003). In this article, we have used 2 approaches to demonstrate reduced nuclear and increased cytoplasmic localization of a key epigenetic molecule, H3K4me3, in affected neurons in AD. The concept of ectopic localization of epigenetic (and other) molecules in a variety of situations is not unique to the present findings. Many studies have demonstrated reduced epigenetic molecules in the nucleus in a variety of situations, for example (Desplats et al., 2011; Kamikawa and Donohoe, 2014; Mastroeni et al., 2009; Ogawa et al., 2003; Thatcher and LaSalle, 2006).

We have previously demonstrated ectopic localization of epigenetic molecules in AD (Mastroeni et al., 2010, 2013) and this demonstration in AD of a reciprocal relationship between cytoplasmic and nuclear localization of an epigenetic molecule in AD confirms and extends the ectopic localization of selected epigenetic molecules in AD. However, ectopic localization of epigenetic molecules is selective because not all epigenetic molecules exhibit this, as shown in Supplementary Fig. 3. The data presented in Supplementary Fig. 2 further indicates that AD and PD differentially affect ectopic localization of H3k4me3 in the substantia nigra although both diseases are considered neurodegenerative. The full consequences of the ectopic presence of selected molecules in the cytoplasm is not yet known, but existing data suggest that their presence in the cytoplasm has consequences for tau hyper-phosphorylation (Facchinetti et al., 2014) and axonal transport (Kim and Casaccia, 2010; Kim et al., 2010), among others.

In addition to the potential inhibition of many cellular processes, the mislocalization of nuclear H3k4me3 has presumptive significance for chromatin structure and transcription (Sims et al., 2007) with obvious consequences for gene expression. A recent integrated analysis of whole-genome DNA methylation and histone modifications reported that H3K4me3 had a major weight in modeling the combined effects of DNA methylation and histone modifications on gene expression (Lou et al., 2014). Large-scale and genome-wide

Author Manuscript

profiling studies of H3K4me3 in the human and mouse genomes further indicate that this modification is preferentially associated with the promoters of active genes (Barski et al., 2007; Bernstein and Kellis, 2005). In fact, definition of loci tagged with H3k4me3 in human cortical neurons was highly enriched in genes in Gene Ontology categories for synaptic transmission, neurite development and neurogenesis (Cheung et al., 2010)—all functions that are affected in AD.

Our ELISA data indicating no change in amount of H3k4me3 in AD (Fig. 1F), but altered intracellular localization (Fig. 1E and D), suggest that the altered transcription in AD of genes affected by H3k4me3 may be due to altered intracellular localization rather than a change in trimethylation of H3 at the k4 site, which stresses the importance of where the protein is being expressed not how much.

Author Manuscript

We have shown that cytoplasmic localization is an early event in Alzheimer's pathophysiology that is a function of Braak stage, increasing from 0% cytoplasmic localization of H3k4me3 in clean ND to nearly 80% in late stage AD. Additionally, this cytoplasmic localization was seen in neurons that did not exhibit any of the tau marks indicative of early Alzheimer cellular pathology, for example (Weaver et al., 2000). The relation of cytoplasmic H3k4me3 to additional indicators of early cellular pathology, such as altered endosomes (Nixon, 2005), mitochondrial dysfunction (Hirai et al., 2001) remains unknown.

Author Manuscript

Although cytoplasmic IR for H3k4me3 was found to colocalize with all 4 tau antibodies we tested, both MC1 and PG5 showed the strongest association with H3k4me3 IR. Previous studies analyzing MC1 and PG5 have demonstrated that the states recognized by these antibodies precede the formation of NFT (Jicha et al., 1999). Because of the fact, that all tau antibodies tested colocalize to some extent suggest that either H3k4me3 association with each tau epitope is a function of binding efficacy at the various epitopes exposed or there may be different intracellular amounts of cytoplasmic H3k4me3 IR associated with each tau mark. Further studies on the ultrastructure and interactions between these select tau antibodies and cytoplasmic H3k4me3 will be a key in determining exactly which conformation and/or phosphorylation states are important in determining the interaction between tau and H3k4me3. Additional studies will also be needed to determine if cytoplasmic H3k4me3 associates with other cytoplasmic inclusions such as TDP-43 or other pathogenic abnormalities.

Author Manuscript

Although several studies have shown that the 3xTg-AD mouse model of AD replicates many of the pathological features associated with AD in human brain (Oddo et al., 2003a, 2003b), it appears that this model does not recapitulate the cytoplasmic localization of H3k4me3 we observed in human. Similar findings were observed in the APP23 mice of AD (data not shown), which indicates that both a nuclear loss of H3k4me3 and the cytoplasmic accumulation may be independent phenomena, although alternative explanations are possible.

4.1. Discussion summary

Our data show increasing cytoplasmic localization of H3k4me3 with increasing Braak stage as well as colocalization of H3k4me3 IR with tau markers of early disease states. Increased cytoplasmic localization of H3k4me3 IR is accompanied by reduced nuclear localization of this molecule in human brain. Our findings in Braak II ND cases show that the ectopic cytoplasmic localization of H3k4me3 precedes the earliest tau marks tested, which indicates that this event may be quite early in the cascade of cell biological events in the progression of AD. The ability of nuclear H3k4me3 to alter the accessibility of transcription factors and transcription complexes to the transcription start site of synaptic genes (Cheung et al., 2010; Cruceanu et al., 2013) could link these findings to reports suggesting an overall decrease in the expression of synaptic genes in AD (Berchtold et al., 2013). The fact that the mouse model showed nuclear loss but not cytoplasmic accumulation suggests that cytoplasmic accumulation in AD may not be a simple matter of accumulation because of inability to access the nucleus but may prove to be a fundamental process with a functional role. We propose that the future of AD lies in epigenetic mechanisms that influence the expression of key molecules in AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona for the provision of human brain samples. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901, and 1001 to the Arizona Parkinson's Disease Consortium), and the Michael J. Fox Foundation for Parkinson's Research. This work was supported by NIH R01 AG036400 and AARC DHS Award FY 2012 to Paul D. Coleman; NIRG-14-321390 and ADHS14-080000 FY 2015 to Diego Mastroeni; and NIH R01 AG037637 to Salvatore Oddo. Diego Mastroeni discovered the finding, wrote the article, and contributed to the immunohistochemistry and statistical analysis; Elaine Delvaux contributed to the experimental design and statistics; Jennifer Nolz contributed to the immunohistochemistry on human and mice, cell counting and/or quantification; Yuyan Tan contributed to the immunohistochemistry and data analysis; Andrew Grover contributed to the manuscript preparation and data analysis; Salvatore Oddo contributed to the manuscript preparation and mice preparation for immunohistochemistry; Paul D. Coleman contributed to the manuscript preparation, experimental design and statistics.

References

- Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* 2002; 103:26–35. [PubMed: 11837744]
- Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res.* 2002; 21:381–395. [PubMed: 21321607]
- Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell.* 2007; 129:823–837. [PubMed: 17512414]
- Beach TG, Sue LI, Walker DG, Roher AE, Lue L, Vedders L, Connor DJ, Sabbagh MN, Rogers J. The Sun Health Research Institute Brain Donation Program: description and experience, 1987–2007. *Cell Tissue Bank.* 2008; 9:229–245. [PubMed: 18347928]

- Berchtold NC, Coleman PD, Cribbs DH, Rogers J, Gillen DL, Cotman CW. Synaptic genes are extensively downregulated across multiple brain regions in normal human aging and Alzheimer's disease. *Neurobiol Aging*. 2013; 34:1653–1661. [PubMed: 23273601]
- Berger SL. Histone modifications in transcriptional regulation. *Curr Opin Genet Dev*. 2002; 12:142–148. [PubMed: 11893486]
- Bernstein BE, Kellis M. Large-scale discovery and validation of functional elements in the human genome. *Genome Biol*. 2005; 6:312. [PubMed: 15774039]
- Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol*. 1991; 82:239–259. [PubMed: 1759558]
- Braak H, Del Tredici K. The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathol*. 2011; 121:171–181. [PubMed: 21170538]
- Braak H, Thal DR, Ghebremedhin E, Del Tredici K. Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J Neuropathol Exp Neurol*. 2011; 70:960–969. [PubMed: 22002422]
- Cheung I, Shulha HP, Jiang Y, Matevosian A, Wang J, Weng Z, Akbarian S. Developmental regulation and individual differences of neuronal H3K4me3 epigenomes in the prefrontal cortex. *Proc Natl Acad Sci U S A*. 2010; 107:8824–8829. [PubMed: 20421462]
- Cruceanu C, Freemantle E, Alda M, Rouleau GA, Turecki G. Epigenetic regulation of synapsin genes in mood disorders. *Neuropsychopharmacology*. 2013; 38:239–241. [PubMed: 23147482]
- Davies P. Characterization and use of monoclonal antibodies to tau and paired helical filament tau. *Methods Mol Med*. 2000; 32:361–373. [PubMed: 21318532]
- Desplats P, Spencer B, Coffee E, Patel P, Michael S, Patrick C, Adame A, Rockenstein E, Masliah E. Alpha-synuclein sequesters Dnmt1 from the nucleus: a novel mechanism for epigenetic alterations in Lewy body diseases. *J Biol Chem*. 2011; 286:9031–9037. [PubMed: 21296890]
- Espada J, Esteller M. Epigenetic control of nuclear architecture. *Cell Mol Life Sci*. 2007; 64:449–457. [PubMed: 17221334]
- Facchinetti P, Dorard E, Contremoulins V, Gaillard MC, Deglon N, Sazdovitch V, Guihenneuc-Jouyaux C, Brouillet E, Duyckaerts C, Allinquant B. SET translocation is associated with increase in caspase cleaved amyloid precursor protein in CA1 of Alzheimer and Down syndrome patients. *Neurobiol Aging*. 2014; 35:958–968. [PubMed: 24262202]
- Fraga MF, Esteller M. Epigenetics and aging: the targets and the marks. *Trends Genet*. 2007; 23:413–418. [PubMed: 17559965]
- Gilbert N, Thomson I, Boyle S, Allan J, Ramsahoye B, Bickmore WA. DNA methylation affects nuclear organization, histone modifications, and linker histone binding but not chromatin compaction. *J Cell Biol*. 2007; 177:401–411. [PubMed: 17485486]
- Gupta S, Kim SY, Artis S, Molfese DL, Schumacher A, Sweatt JD, Paylor RE, Lubin FD. Histone methylation regulates memory formation. *J Neurosci*. 2010; 30:3589–3599. [PubMed: 20219993]
- Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci*. 2001; 21:3017–3023. [PubMed: 11312286]
- Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet*. 2003; 33(Suppl):245–254. [PubMed: 12610534]
- Jicha GA, Lane E, Vincent I, Otvos L Jr, Hoffmann R, Davies P. A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. *J Neurochem*. 1997; 69:2087–2095. [PubMed: 9349554]
- Jicha GA, Weaver C, Lane E, Vianna C, Kress Y, Rockwood J, Davies P. cAMP-dependent protein kinase phosphorylations on tau in Alzheimer's disease. *J Neurosci*. 1999; 19:7486–7494. [PubMed: 10460255]
- Kamikawa YF, Donohoe ME. The localization of histone H3K27me3 demethylase Jmjd3 is dynamically regulated. *Epigenetics*. 2014; 9:834–841. [PubMed: 24646476]
- Kim JY, Casaccia P. HDAC1 in axonal degeneration: a matter of subcellular localization. *Cell Cycle*. 2010; 9:3680–3684. [PubMed: 20930523]

- Kim JY, Shen S, Dietz K, He Y, Howell O, Reynolds R, Casaccia P. HDAC1 nuclear export induced by pathological conditions is essential for the onset of axonal damage. *Nat Neurosci.* 2010; 13:180–189. [PubMed: 20037577]
- Lou S, Lee HM, Qin H, Li JW, Gao Z, Liu X, Chan LL, Ki Lam V, So WY, Wang Y, Lok S, Wang J, Ma RC, Tsui SK, Chan JC, Chan TF, Yip KY. Whole-genome bisulfite sequencing of multiple individuals reveals complementary roles of promoter and gene body methylation in transcriptional regulation. *Genome Biol.* 2014; 15:408. [PubMed: 25074712]
- Mastroeni D, Chouliaras L, Grover A, Liang WS, Hauns K, Rogers J, Coleman PD. Epigenetic differences in cortical neurons from a pair of monozygotic twins discordant for Alzheimer's disease. *PLoS One.* 2009; 4:e6617. [PubMed: 19672297]
- Mastroeni D, Grover A, Delvaux E, Whiteside C, Coleman PD, Rogers J. Epigenetic changes in Alzheimer's disease: decrements in DNA methylation. *Neurobiol Aging.* 2010; 31:2025–2037. [PubMed: 19117641]
- Mastroeni D, McKee A, Grover A, Rogers J, Coleman PD. Reduced RAN expression and disrupted transport between cytoplasm and nucleus; a key event in Alzheimer's disease pathophysiology. *PLoS One.* 2013; 8:e53349. [PubMed: 23308199]
- Nixon RA. Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. *Neurobiol Aging.* 2005; 26:373–382. [PubMed: 15639316]
- Oddo S, Caccamo A, Kitazawa M, Tseng BP, LaFerla FM. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. *Neurobiol Aging.* 2003a; 24:1063–1070. [PubMed: 14643377]
- Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A β and synaptic dysfunction. *Neuron.* 2003b; 39:409–421. [PubMed: 12895417]
- Ogawa O, Zhu X, Lee HG, Raina A, Obrenovich ME, Bowser R, Ghanbari HA, Castellani RJ, Perry G, Smith MA. Ectopic localization of phosphorylated histone H3 in Alzheimer's disease: a mitotic catastrophe? *Acta Neuropathol.* 2003; 105:524–528. [PubMed: 12677454]
- Perl DP. Neuropathology of Alzheimer's disease. *Mt Sinai J Med.* 2010; 77:32–42. [PubMed: 20101720]
- Sims RJ 3rd, Millhouse S, Chen CF, Lewis BA, Erdjument-Bromage H, Tempst P, Manley JL, Reinberg D. Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. *Mol Cell.* 2007; 28:665–676. [PubMed: 18042460]
- Thatcher KN, LaSalle JM. Dynamic changes in histone H3 lysine 9 acetylation localization patterns during neuronal maturation require MeCP2. *Epigenetics.* 2006; 1:24–31. [PubMed: 17464364]
- Wang JZ, Xia YY, Grundke-Iqbal I, Iqbal K. Abnormal hyper-phosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. *J Alzheimers Dis.* 2013; 33(Suppl 1):S123–S139. [PubMed: 22710920]
- Weaver CL, Espinoza M, Kress Y, Davies P. Conformational change as one of the earliest alterations of tau in Alzheimer's disease. *Neurobiol Aging.* 2000; 21:719–727. [PubMed: 11016541]

Appendix A. Supplementary data

Supplementary data associated with this article can be found at the online version at <http://dx.doi.org/10.1016/j.neurobiolaging.2015.08.017>.

Fig. 1.

H3k4me3 IR in the CA1 of the hippocampus of clean, no-pathology, nondemented (ND) control (A), Braak II control (B), and Braak IV Alzheimer's case (C). No-pathology control cases show strong nuclear H3k4me3 IR (A, inset) but no cytoplasmic accumulation. Braak II ND cases, however, occasionally do show diffuse cytoplasmic H3k4me3 IR (B, see inset). Conversely, AD case shows robust cytoplasmic H3k4me3 IR more so than the nuclear compartment (C, see inset). AD cases were more often observed to express cytoplasmic H3k4me3 IR if the nuclei were displaced (B, see inset and red arrows). Normal nuclear distribution in the same AD cases revealed significant nuclear loss but only moderate cytoplasmic IR (B, red arrowheads). (D) Bar graph shows the mean signal of 200 neurons from CA1 of the hippocampus. Neurons were evaluated by delineating the nucleus (dashed circle) from cytoplasm (solid black line), see inset on H3k4me3 micrograph for details. The bar graph represents the mean intensity value of each immunoreactive location (i.e., nuclear or cytoplasmic). Western blot analysis of nuclear and cytoplasmic preparations from

homogenates (middle temporal gyrus [MTG]) from Braak IV AD and Braak II control confirms immunohistochemical quantification data (E). Total H3k4me3 IR detected by ELISA, however, using the same antibody used for immunohistochemistry and Western blotting shows that globally there is no significant difference between Braak IV AD cases and Braak II ND cases (F). These data stress the importance of multilevel examination to determine both protein concentration and intracellular localization. *Indicates a significant difference compared to control, 2-tailed t test $p < 0.05$. Error bars represent the standard error of the mean. ** $p < 0.001$. Abbreviations: AD, Alzheimer's disease; ELISA, enzyme-linked immunosorbent assay; H3k4me3, histone 3 trimethylation on lysine 4; IR, immunoreactivity. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 2.

Mean percent of cells with cytoplasmic H3k4me3 immunoreactivity in Braak 0 and II–IV controls and Braak IV and Braak VI AD cases. Two hundred individual neurons from CA1 were determined if (1) they had cytoplasmic immunoreactivity or (2) did not. Four cases per Braak stage were used, totaling 600 individual neurons per Braak stage. Error bars represent the standard error of the mean. Abbreviations: AD, Alzheimer's disease; H3k4me3, histone 3 trimethylation on lysine 4; ND, nondemented.

Fig. 3.

Cytoplasmic accumulation of H3k4me3 precedes pretangle antibodies. Serial, Braak 0 sections show no immunoreactivity for either early tau markers PG5 or MC1 but show strong nuclear labeling for H3k4me3 and no cytoplasmic immunoreactivity (IR). Braak II ND cases do show modest cytoplasmic IR for H3k4me3 (bottom right panel, see inset). Braak IV AD sections, however, show strong cytoplasmic IR for MC1, PG5, and H3k4me3 (right column). The data from Braak 0 cases show that cytoplasmic accumulation of H3k4me3 is not an aging effect. Braak II control cases show cytoplasmic H3k4me3 before early tau markers. Alzheimer's cases were used as positive controls. $*p < 0.01$. Abbreviations: AD, Alzheimer's disease; H3k4me3, histone 3 trimethylation on lysine 4; ND, nondemented.

Fig. 4.

Confocal micrographs of neurons in the CA1 hippocampus; green fluorophore (H3k4me3) and red fluorophore (selected tau antibodies). Colocalization analysis reveals subtle overlap with neurofibrillary tangles marker PHF-1 (A), modest overlap with CP13 (C) and robust colocalization with early markers of tau aggregation MC1 (B) and PG5 (D) in Braak IV AD and Braak IV ND cases. Abbreviations: AD, Alzheimer's disease; H3k4me3, histone 3 trimethylation on lysine 4; ND, nondemented. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 5.

H3k4me3 IR of the hippocampus of the 3xTg-AD mouse model of AD. Micrographs show similar patterns of nuclear loss in a disease-dependent manner (by age). Although both AD and 3xTg-AD mouse model show nuclear loss in the hippocampus, the most notable region affected in mice was in the CA3 (see D, arrow and E for quantitative analysis). The loss of IR shifts from CA3 to CA2 followed by only modest changes in the CA1 by 19 months (D and E). Although nuclear loss was evident by 19 months, cytoplasmic accumulation was absent in all regions and all ages analyzed. *Indicates a significant difference compared to control, 2-tailed t test $p < 0.05$. ** $p < 0.001$, *** $p < 0.0001$. Error bars represent the standard

error of the mean. Abbreviations: AD, Alzheimer's disease; H3k4me3, histone 3 trimethylation on lysine 4; IR, immunoreactivity.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Characteristics of cases used for quantitative and qualitative assessment

Gender	Age expired	PMI	Control	AD	PD	Braak score
Male	78	4.5	Yes	No	No	0
Male	86	3	Yes	No	No	0
Male	82	2.75	Yes	No	No	0
Female	86	4.83	Yes	No	No	0
Male	83	4.33	No	No	Yes	0
Male	85	4	No	No	Yes	I
Male	74	3	No	No	Yes	I
Male	72	2	No	No	Yes	I
Female	90	3	Yes	No	No	II
Male	86	2.5	Yes	No	No	II
Male	86	2.66	Yes	No	No	II
Female	90	2.5	Yes	No	No	II
Male	83	2.16	Yes	No	No	III
Female	96	3	Yes	No	No	III
Male	74	2.75	Yes	No	No	III
Female	96	1.5	Yes	No	No	III
Male	80	2.83	Yes	No	No	III
Male	80	2.56	Yes	No	No	IV
Female	88	2.66	Yes	No	No	IV
Male	81	3	Yes	No	No	IV
Female	62	3.5	Yes	No	No	IV
Male	78	2	Yes	No	No	IV
Male	90	2.66	Yes	No	No	IV
Male	78	2.16	No	Yes	No	IV
Male	97	3	No	Yes	No	IV
Male	78	3	No	Yes	No	IV
Male	80	3.33	No	Yes	No	IV
Female	91	1.83	No	Yes	No	IV

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Gender	Age expired	PMI	Control	AD	PD	Braak score
Female	77	4	No	Yes	No	IV
Female	96	4	No	Yes	No	IV
Female	86	2.5	No	Yes	No	IV
Female	91	4.5	No	Yes	No	IV
Male	85	5.75	No	Yes	No	IV
Female	75	3.16	No	Yes	No	VI
Male	70	1.83	No	Yes	No	VI
Male	76	2.83	No	Yes	No	VI
Female	86	1.83	No	Yes	No	VI
Female	88	3.5	No	Yes	No	VI
Male	95	2.83	No	Yes	No	VI
Female	82	3	No	Yes	No	VI
Female	103	3.5	No	Yes	No	VI

Key: AD, Alzheimer's disease; PD, Parkinson's disease; PMI, postmortem interval.

Table 2

Antibody and dilutions

Antibody	Host	Dilution	Source/catalogue#
H3k4me3	Rabbit	1/1000	Cell Signaling/9727
H3k4me3 peptide	Rabbit	1/500	Cell Signaling/1064
Total H3	Rabbit	1/2000	Cell Signaling/9715
H3k9me2	Rabbit	1/1000	Cell Signaling/9753
Total H4	Rabbit	1/1000	Cell Signaling/2935
Acetylated H4k12	Rabbit	1/1000	Cell Signaling/2591
Beta actin	Mouse	1/3000	Sigma/A1978
Lamin A	Mouse	1/2000	Sigma/SAB4200420
PHF-1	Mouse	1/1000	Gift P. Davies
CP13	Mouse	1/1000	Gift P. Davies
Pg5	Mouse	1/1000	Gift P. Davies
MC1	Mouse	1/1000	Gift P. Davies

Key: H3k4me3, histone 3 trimethylation on lysine 4.