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Epigenetic changes in Alzheimer's disease: Decrements in DNA methylation

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Abstract

DNA methylation is a vital component of the epigenetic machinery that orchestrates changes in multiple genes and helps regulate gene expression in all known vertebrates. We evaluated immunoreactivity for two markers of DNA methylation and eight methylation maintenance factors in entorhinal cortex layer II, a region exhibiting substantial Alzheimer's disease (AD) pathology in which expression changes have been reported for a wide variety of genes. We show, for the first time, neuronal immunoreactivity for all 10 of the epigenetic markers and factors, with highly significant decrements in AD cases. These decrements were particularly marked in PHF1/PS396 immunoreactive, neurofibrillary tangle-bearing neurons. In addition, two of the DNA methylation maintenance factors, DNMT1 and MBD2, have been reported also to interact with ribosomal RNAs and ribosome synthesis. Consistent with these findings, DNMT1 and MBD2, as well as p66α, exhibited punctate cytoplasmic immunoreactivity that co-localized with the ribosome markers RPL26 and 5.8 s rRNA in ND neurons. By contrast, AD neurons generally lacked such staining, and there was a qualitative decrease in RPL26 and 5.8 s rRNA immunoreactivity. Collectively, these findings suggest epigenetic dysfunction in AD-vulnerable neurons.

Keywords: Epigenetics; DNA methylation; Alzheimer's disease; Neuron; Ribosome

1. Introduction

Gene expression in the Alzheimer's disease (AD) brain has been shown to be altered in a wide variety of reports (Robinson et al., 1994; Loring et al., 2001; Dunckley et al., 2006; Weeraratna et al., 2007; Liang et al., 2008a,b,c), including a recent large-scale expression array study of single cell laser-captured entorhinal cortex layer II neurons (Dunckley et al., 2006). Multiple physiologic and molecular pathways are affected, including energy metabolism (Liang et al., 2008c) inflammation (Loring et al., 2001; Weeraratna et al., 2007) and aberrant cell cycle events (Arendt, 2000; Bowser and Smith, 2002), among others. Although individual pathogenic factors such as amyloid β peptide (Aβ) and tau phosphorylation are clearly critical links, no over-arching principle to explain the consistency, extent, and breadth of the gene expression, physiologic, and molecular changes reported in AD has received consensus acceptance. Epigenetic mechanisms such as histone modification (Mclachlan et al., 1984), binding of non-histone proteins, and DNA methylation (Adcock et al., 2007; Suzuki and Bird, 2008) are capable of modulating coordinate expression of large numbers of genes across many different pathways, and may therefore warrant investigation for their potential role in AD pathogenesis.

DNA methylation is a highly conserved process that has been implicated in many different modalities of gene expression. The factors responsible for the methylation process are a family of DNA methyltransferases that have been shown to catalyze the transfer of a methyl group to single-stranded DNA using *S*-adenosyl methionine as the methyl donor. The recognition sequence for the mammalian DNA methyltransferase is relatively invariant, with nearly all cytosine methylations occurring on 5′-C-p-G-3′ (CpG) (Bird, 1986, 1992). There are four known active DNA methyltransferases

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in mammals, DNMT1, DNMT2, DNMT3A and DNMT3B. Of these, DNMT1 is the most abundant in mammalian cells. DNMT1 has been reported to be a key player in maintaining methylation in somatic cells, and loss of this enzyme has been shown to lead to nuclear disorganization, increased histone acetylation, and apoptosis (Chan et al., 2001; Fan et al., 2001; Jackson et al., 2004; Milutinovic et al., 2004; Espada et al., 2007).

Once methylation has occurred, methylation stability is maintained by the binding of specific complexes, MeCP1, to methylated regions of DNA. MeCP1 is not bound directly to methylated DNA, but rather to a single methyl-CpG-binding domain protein, MBD2. The resulting MeCP1/MBD2 complex is composed of 10 known proteins that include the complete nucleosome remodeling and histone deacetylase (NuRD) core, as well as MBD2. This group of proteins, in conjunction with CDK2AP1 (Doc1), make up a complex capable of nucleosome remodeling and histone deacetylation (Feng and Zhang, 2001, 2003).

Because methylation and methylation maintenance factors can orchestrate changes in expression of a wide range of genes (Ashraf and Ip, 1998; Nan et al., 1998; Fujita et al., 1999; Ng et al., 1999; Feng and Zhang, 2001; Adcock et al., 2007; Suzuki and Bird, 2008), we hypothesized that alterations in methylation and methylation stability might provide an over-arching mechanism that could help explain expression differences in the thousands of genes that are reportedly altered in AD (Robinson et al., 1994; Loring et al., 2001; Dunckley et al., 2006; Liang et al., 2007, 2008a,b,c; Weeraratna et al., 2007). Here, we report highly significant decrements in immunoreactivity for two markers of DNA methylation and eight DNA methylation maintenance factors in AD neurons of entorhinal cortex layer II, one of the most consistently vulnerable brain regions to AD pathology (Braak et al., 1993; Kordower et al., 2001).

2. Methods

2.1. Subjects and brain samples

Brain tissue was obtained through the Sun Health Research Institute Brain and Body Donation Program (Sun City, AZ). Specimens were collected under IRB-approved protocols and informed consents that permitted use of the samples for research by the investigators. Cases included in the study had received antemortem evaluation by board-certified neurologists and neuropsychologists, as well as postmortem evaluation by a board-certified neuropathologist. Evaluations and diagnostic criteria followed consensus guidelines for National Institute on Aging Alzheimer's Disease Centers. Diagnoses of patient condition included AD ($N = 20$) and cognitively and neurologically normal for age (ND) ($N = 20$). AD patients taking conventional AD therapeutics were not excluded from the study. At expiration, subject ages ranged from 60 to 97 years, with a mean of 79.9 ± 1.3 years. Postmortem intervals for the subjects averaged 2 h 40 min $\pm$ 12 min. Subject age, gender, and postmortem interval were well matched between the experimental groups, and there were no significant between-groups differences on any of these variables. Because neurons of entorhinal cortex layer II, which form clusters or "islands", are among the earliest and most consistently impacted neurons in AD brain (26), this brain region was the focus for all experiments.

2.2. Immunohistochemistry

Temporal cortex was sliced axially into 1-cm thick slabs, immersion fixed for 48 h in buffered 4% paraformaldehyde at 4 °C, washed extensively in phosphate buffer (PB), and cryoprotected in ethylene glycol and glycerol. The slabs were then sectioned at 40 μm a freezing cryostat. Free-floating sections at the level of the entorhinal cortex were stored in freezing solution (glycol/glycerol/PB) at −20 °C until required for experiments. Tissue sections used for bright field microscopy were immunoreacted using the avidin–biotin complex/diaminobenzidine (DAB) method. Briefly, tissues were washed 2×, blocked in 1% hydrogen peroxide for 45 min, washed 3×, blocked in 3% bovine serum albumin (BSA) for 1 h, washed 2×, and incubated at 4 °C overnight in primary antibody solutions containing 0.25% BSA. Unless otherwise stated, all washes were with 1× PBS Triton (PBST). Available information about the antibodies is given in Table 1. After incubation with primary antibody, sections were washed 3×, incubated in biotinylated, species-specific secondary antibodies (Vector) for 2 h, washed 3×, and incubated in avidin–biotin complex (Pierce) for 1 h. Following incubation with secondary antibody, the sections were washed 3×, once in PBST and twice in 0.05 M Tris buffer, then exposed to DAB solution containing 125 μl of 5 mg/ml DAB (Sigma), 11.125 ml 50 mM Tris buffer pH 7.6, and 500 μl saturated nickel ammonium sulfate. Incubations during chromagen development were no longer than 10 min, and were followed by two quick rinses in 50 mM Tris to stop the reaction. Finally, the sections were dried, taken through graded alcohols, de-fatted in Neoclear (EMD), and mounted with Permount (Pierce). AD and ND sections were immunoreacted simultaneously using netwells in well-less plates. For fluorescence microscopy, the sections were washed 3×, blocked with either 3% normal goat serum or 3% BSA, and incubated for 2 h. The sections were then washed 2×, incubated in primary antibody in 0.25% BSA at 4 °C overnight, washed again, and incubated in species-specific, fluorophore-conjugated secondary antibodies (Molecular Probes) at room temperature for 2 h. After a final wash, the sections were mounted, taken through Sudan Black to reduce autofluorescence, and coverslipped with Vectashield mounting media (Vector). Deletion of primary antibody or incubation with pre-immune serum resulted in abolition of specific immunoreactivity in all cases (data not shown). Adjacent serial sections were stained with cresyl violet for cell layer identification and verification that the island