Alzheimer's & Dementia 13 (2017) 674-688

Featured Article

A genome-wide profiling of brain DNA hydroxymethylation in Alzheimer's disease

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Abstract

Introduction: DNA methylation is a key epigenetic mechanism in brain aging and Alzheimer's disease (AD). The newly discovered 5-hydroxymethylcytosine mediates DNA demethylation, is highly abundant in the brain, and is dynamically regulated by life experiences. However, little is known about its genome-wide patterns and potential role in AD.

Methods: Using a genome-wide capture followed by high-throughput sequencing, we studied the genome-wide distribution of 5-hydroxymethylcytosine at specific genomic loci in human AD brain and identified differentially hydroxymethylated regions (DhMRs) associated with AD pathology.

Results: We identified 517 DhMRs significantly associated with neuritic plaques and 60 DhMRs associated with neurofibrillary tangles. DNA hydroxymethylation in gene bodies was predominantly positively correlated with *cis*-acting gene expression. Moreover, genes showing differential hydroxymethylation were significantly enriched in neurobiological processes and clustered in functional gene ontology categories.

Discussion: Our results reveal a critical role of DNA hydroxymethylation in AD pathology and provide mechanistic insight into the molecular mechanisms underlying AD.

Keywords: Alzheimer's disease; Postmortem brain; DNA hydroxymethylation; Genome-wide association; Epigenetics

1. Introduction

Alzheimer's disease (AD) is characterized clinically by progressive cognitive decline and histologically by neuritic plaques (NPs), neurofibrillary tangles (NFTs), and loss of neurons in the brain. Epigenetic factors mediate the effects of age and environmental factors independently of DNA sequence, and epigenetic dysregulation has been implicated in brain aging and neurodegenerative disorders including AD [1]. Elucidating epigenetic pathways is likely to provide mechanistic insight into disease etiology and holds promise for discovering novel strategies for early detection and therapeutic intervention at preclinical stages of the disease.

DNA methylation on the fifth carbon of the cytosine base (5-methylcytosine, 5mC) is the most extensively studied epigenetic modification that is essential for neurogenesis [2], learning and memory [3], and synaptic plasticity [4]. Altered levels of 5mC have been associated with AD diagnosis and numerous neuropathologic phenotypes [5–8], although results are somewhat conflicting [5,9–11]. Notably, in a large collection of postmortem human brains, our group has recently identified an association between altered levels of 5mC with the burden of AD pathology [12–15].

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http://dx.doi.org/10.1016/j.jalz.2016.10.004
1552-5260/

The newly discovered 5-hydroxymethylcytosine (5hmC) is an oxidative product of 5mC catalyzed by the ten-eleven translocation family of proteins. This conversion is an important mechanism underlying the active demethylation of DNA [16]. 5hmC acts as an intermediate in DNA demethylation and also serves as a stable epigenetic mark during the development of disease [17]. Studies of mouse brain indicated that 5hmC is particularly enriched in the brain [9,18–20], dynamically regulated during neurodevelopment [21–23], and accumulates with age across the lifespan [21,23–26]. Unlike 5mC, which is often located in CpG-rich regions (e.g., promoters), 5hmC occurs primarily in gene bodies and exon-intron boundaries. As with 5mC, 5hmC levels vary substantially between different cell types and tissues, and its enrichment in gene bodies positively influences gene expression in the human brain [27]. These findings suggest that 5hmC represents a new dimension of epigenetic regulation that may play an important role in brain aging and neurodegenerative disorders. However, there is little research examining the genome-wide patterns of 5hmC in human AD brain and its association with AD pathology in human populations. Here, we report findings from a genome-wide profiling of 5hmC in human postmortem brain tissue from a community-based cohort of aging and dementia with brain donation at the time of death with the goal of identifying differentially hydroxymethylated regions (DhMRs) associated with quantitative measures of neuropathologic burden on AD.

2. Methods

2.1. Subject population and postmortem brain sample

Postmortem human dorsolateral prefrontal cortex tissue samples (N = 30) were obtained from deceased participants in two community-based ongoing prospective cohort studies of older individuals: the Religious Orders Study and the Rush Memory and Aging Project. Detailed study design and assessment procedures have been described previously [28,29]. In brief, Religious Orders Study recruited more than 1300 older Catholic priests, nuns, and brothers from across the United States. Memory and Aging Project recruited more than 1800 older men and women from across the Chicagoland area. All participants were free of dementia at the time of enrollment and agreed to annual clinical evaluations and brain donation on death. The clinical evaluation included detailed neurologic examination and clinical classification of dementia and AD. At the time of death, a summary diagnosis of AD was made based on review of all clinical data without access to the postmortem data [30]. Both studies were approved by the institutional review board of Rush University Medical Center. Written informed consent was obtained from all subjects, followed by an Anatomic Gift Act for organ donation.

2.2. Assessment of neuropathologic phenotypes

Detailed procedures for postmortem brain examination and neuropathologic phenotyping have been described previously [31,32]. Briefly, Bielschowsky silver stain was used to visualize NP and NFTs in the frontal, temporal, parietal, and entorhinal cortices, and the hippocampus. The quantitative NP and NFT burden was based on counts of the lesions in the maximum density in each region, as described [33,34].

2.3. Genome-wide profiling of brain DNA hydroxymethylome

2.3.1. 5hmC-capture sequencing

Genomic DNA was isolated from 10 mg of frozen dorsolateral prefrontal cortex with proteinase K digestion. 5hmC enrichment was performed using previously described selective chemical labeling technique [25]. This method used the T4 bacteriophage β-glucosyltransferase to specifically modify 5hmC residues by adding glucose moiety to 5hmC. 5hmC-captured libraries were generated following the Illumina protocol for "Preparing Samples for ChIP Sequencing of DNA." We used 25 ng of input genomic DNA or 5hmC-captured DNA to initiate the protocol. DNA fragments were gel-purified after adapter ligation. Polymerase chain reaction–amplified DNA libraries were quantified on an Agilent 2100 Bioanalyzer and diluted to 6 to 8 pM for cluster generation and sequencing. We performed 38-cycle single-end sequencing generation on Illumina HiSeq 2000 to obtain 5hmC-enriched DNA fragment sequence.

2.3.2. Sequence alignment, 5hmC quantification, and distribution

FASTQ files were aligned to the human genome (hg19) with Bowtie, retaining only nonduplicate genomic matches with no more than two mismatches in the first 25 bp [35]. Model-based analysis of ChIP-Seq (MACS) software was used to estimate the level of 5hmC enrichment in each sample by directly comparing to the input DNA (effective genome size = 1.87×10^9, tag size = 38, and bandwidth = 200). Unique, nonduplicate reads were counted in 10 kb bins and normalized to the total number of nonduplicate reads. Genome-wide patterns of 5hmC were evaluated by counting mapped reads per 10 kb bin, which were then normalized to sequencing coverage.

2.4. Statistical and bioinformatics analysis

2.4.1. DhMR analysis

To identify differentially DhMRs associated with AD pathology, we separately constructed zero-inflated negative binomial regression models for quantitative measures of NP or NFTs, adjusting for potential confounding factors including age at the time of death, sex, education level,