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Brief communication

Cross-region reduction in 5-hydroxymethylcytosine in Alzheimer's disease brain

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ABSTRACT

Epigenetic processes play a key role in the central nervous system and altered levels of 5-methylcytosine have been associated with a number of neurologic phenotypes, including Alzheimer's disease (AD). Recently, 3 additional cytosine modifications have been identified (5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine), which are thought to be intermediate steps in the demethylation of 5-methylcytosine to unmodified cytosine. Little is known about the frequency of these modifications in the human brain during health or disease. In this study, we used immunofluorescence to confirm the presence of each modification in human brain and investigate their cross-tissue abundance in AD patients and elderly control samples. We identify a significant AD-associated decrease in global 5-hydroxymethylcytosine in entorhinal cortex and cerebellum, and differences in 5-formylcytosine levels between brain regions. Our study further implicates a role for epigenetic alterations in AD.

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease affecting >5.4 million adults in the US and contributing significantly to the global burden of disease (Thies and Bleiler, 2011). Given the high heritability estimates for AD (Gatz et al., 2006), considerable effort has focused on understanding the role of genetic variation in disease etiology, although it has been recently speculated that epigenetic dysfunction is also likely to be important (Lunnon and Mill, 2013).

Epigenetics refers to the reversible regulation of various genomic functions occurring independently of DNA sequence, with cytosine methylation being the best-understood and most stable epigenetic modification modulating the transcription of mammalian genomes. Recent studies have identified global- and site-specific alterations in 5-methylcytosine (5-mC) levels in AD brain (Bakulski et al., 2012; Mastroeni et al., 2009, 2010; Rao et al., 2012).

A number of additional cytosine modifications have recently been described. 5-hydroxymethylcytosine (5-hmC) has been shown to be enriched in brain (Khare et al., 2012), suggesting it may play an important role in neurobiological phenotypes and disease. Importantly, current approaches based on sodium bisulfite converted DNA are unable to distinguish between 5-mC and 5-hmC (Nestor et al., 2010). 5-hmC is believed to be an intermediate step in the demethylation of 5-mC to unmodified cytosine by the oxidation of 5-mC by ten eleven translocation

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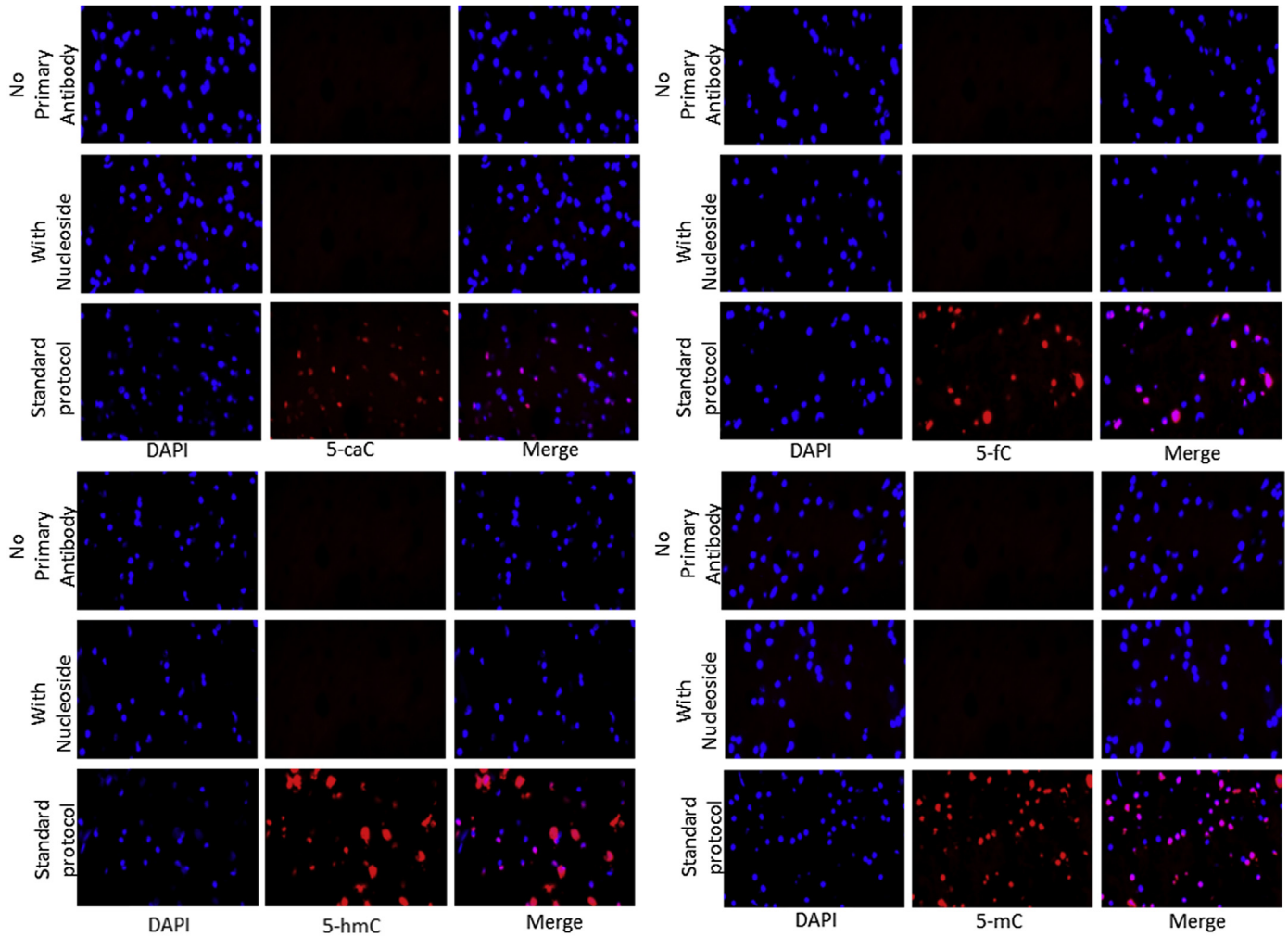

Fig. 1. Immunofluorescence demonstrating the specificity of the antibodies used in this study. At the concentrations used no staining was observed in either the no primary antibody control, or the nucleoside control. Staining of tissue (in absence of controls) was co-localized with 4′,6-diamidino-2-phenylindole (DAPI) demonstrating nuclear staining.

proteins (Tahiliani et al., 2009). 5-hmC is thought to play a specific role in transcriptional regulation as it is recognized by key proteins that do not recognize 5-mC (Jin et al., 2010), has a distinct genomic distribution to 5-mC (being predominantly found in gene promoters and gene bodies and rarely in intergenic regions [Jin et al., 2011; Stroud et al., 2011]), is more abundant in constitutive exons than alternatively spliced ones (Khare et al., 2012) and has a lower affinity to methyl-binding proteins than 5-mC (Hashimoto et al., 2012). 5-hmC appears to be present in all tissues, although at differing levels, with the highest levels observed in brain (Li and Liu, 2011) with enrichment in genes involved in synapse-related functions (Khare et al., 2012); in contrast the distribution of 5-mC appears to be relatively uniform across tissues (Globisch et al., 2010). It has been suggested that although some hydroxymethylated-CpG loci are stable during aging, others are more dynamically altered (Szulwach et al., 2011).

In 2011, two additional cytosine modifications were described in mouse embryonic stem cells and somatic tissues (Inoue et al., 2011; Ito et al., 2011). 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) were found to result from the further oxidation of 5-hmC by ten eleven translocation proteins (Ito et al., 2011), suggesting that these modifications represent further steps along the demethylation pathway (Inoue et al., 2011). Although little is known about their functional role and prevalence in the healthy genome, recent studies have mapped 5-fC to gene regulatory elements, namely poised enhancers and CpG island promoters (Raiber et al., 2012; Song et al., 2013).

The aim of the present study was to investigate the relative abundance of the four described cytosine modifications across two distinct anatomic brain regions (entorhinal cortex [EC] and cerebellum [CER]) in tissue obtained from AD cases and elderly controls.

2. Methods

2.1. Subjects and sample preparation

Formalin-fixed tissue punches from the EC and CER were obtained from AD cases (n = 13) and cognitively normal elderly control (CTL) subjects (n = 8) from the MRC London Neurodegenerative Diseases Brain Bank (http://www.kcl.ac.uk/iop/depts/cn/research/MRC-London-Neurodegenerative-Diseases-Brain-Bank/MRC-London-Neurodegenerative-Diseases-Brain-Bank.aspx). Sample demographics are shown in Supplementary Table 1.