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Identifying microRNAs related to Alzheimer's disease from differential methylation signatures

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ABSTRACT

Understanding the pathophysiology of neurodegenerative diseases like Alzheimer's disease (AD) is still a challenge because of the many layers of complexity that involves gene regulation and control. MicroRNAs (miRNAs) are a kind of regulatory molecules that are responsible for the precise control of genes through translational inhibition or mRNA degradation. DNA methylation on the other hand, is a dynamic chemical modification primarily on the cytosine of a CG dinucleotide. These modifications are mostly found in the promoter regions of genes and aid in their tissue-specific silencing. Abnormal methylation patterns can therefore give rise to unwanted gene expression and silencing leading to a disease phenotype. Our study is aimed to pinpoint key miRNAs that might bear significant relation to the cause of AD, through the identification of differential methylation patterns in AD subjects against the normal individuals. By studying the differential methylation signatures, our analyses revealed 19 miRNAs that regulate genes which are directly or indirectly related to the disease manifestation.

1. Introduction

MicroRNAs (miRNAs) are short non-coding RNA molecules of length ~22 nt that bind to complementary regions of mRNAs and post-transcriptionally repress them through the formation of RNA induced silencing complex (RISC) (Ambros et al., 2003). Anomalous expression of miRNAs is hence responsible for various disease phenotypes ranging from tumorigenesis to neurodegeneration (Li and Kowdley, 2012). A single miRNA can silence the expression of several genes and conversely a single gene can also be silenced by the cooperative action of several miRNAs (Lewis et al., 2003). The role of miRNAs in neuron development and function was first exposed in *C. elegans* (Johnston and Hobert, 2003) and was later implicated in mammalian central nervous system (CNS) (Kosik and Krichevsky, 2005), and various neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. Here, we are concerned about recognizing miRNAs related to AD, which is a progressive age-related dementing disorder principally characterized by the progressive loss of memory and cognitive abilities (Coyle et al., 1983). This occurs due to the accumulation of extracellular senile plaques (SP) formed of amyloid-beta (Aβ) proteins and neurofibrillary tangles (NFT) formed of degenerated neurons in various regions of the brain (Hardy and Selkoe, 2002). For example, entorhinal cortex of the brain is characterized by early susceptibility to AD, whereas the regions like cerebellum remain unaffected from amyloid-beta plaques and neurofibrillary tangles (Wenk, 2003). The extent of this disease is so alarming that a report from the US reveals an increase of 68% of deaths by AD in between 2000 and 2010, although the actual figure is expected to be much higher (Association, 2014).

Most cases of Alzheimer's disease are sporadic AD, which develop due to complex environmental and genetic differences (Ertekin-Taner, 2007). Genetic polymorphism of over 500 genes is thought to be the cause of sporadic AD but only the inheritance of the ε4 allele of the apolipo protein E (APOE) is validated (Maes et al., 2009). The rare form of AD is a familial form of autosomal dominant inheritance and is known as early onset familial Alzheimer's disease. Most of autosomal dominant familial AD is due to mutations in one of three genes, namely amyloid precursor protein (APP) gene, presenilin-1 (PSEN1) and presinilin-2 (PSEN2). Mutations in these genes result in the increase of a small protein called Aβ42, which is the main component of senile plaques (Maes et al., 2009).

Plaques are formed when specific neuronal membrane proteins called amyloid precursor protein (APP) are abnormally processed. Generally, APP is proteolyzed by the enzymes α-secretase and γ-secretase successively. The cleavage by α-secretase creates Aβ40 proteins which are soluble. In unusual conditions, the APP protein is first cleaved by an enzyme called β-secretase (β-site APP cleaving enzyme 1, BACE1) followed by γ-secretase complex (PSEN1 and PSEN2), resulting in the release of short fragments called Aβ42 which are insoluble. This

Abbreviation: miRNA, microRNA; AD, Alzheimer's disease; RISC, RNA induced silencing complex; CNS, central nervous system; SP, senile plaques; Aß, amyloid-beta; NFT, neurofibrillary tangles; GWAS, genome-wide association studies; EWAS, epigenome-wide association studies; TFBS, transcription factor binding site; TF, transcription factor; DMS, differential methylation signature; CREB, cAMP response element binding; FFL, feed forward loop.

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insoluble Aβ42 combines to form the senile plaques that accumulate in the cortex of the brain causing early AD progression (O'Brien and Wong, 2011).

Another characteristic of the AD brain is the formation of NFTs. In normal case, the skeleton of the neuron is made of microtubules that are stabilized by normal tau protein. In AD, the tau proteins become defective due to hyperphosphorylation. This causes their detachment from the microtubules, thereby destabilizing the skeletal structure of the neurons (O'Brien and Wong, 2011). As a result, the neurons degenerate and the connections between them are lost. The defective tau proteins also form filaments, which accumulate within the neurons to create neurofibrillary tangles which ultimately cause the death of the neurons. NFTs first appear in the region called the hippocampus of the brain, an area responsible for memory and learning (O'Brien and Wong, 2011). The progression of NFT corresponds to the symptoms of AD like causing problems in memory and cognitive disabilities, such as problems of speech, recognition and incapacity to perform gestures.

Genome-wide association studies (GWAS) are currently helping to uncover many AD associated genes (Lambert et al., 2013), however, their regulation and the underlying molecular pathways that contribute to the condition still remain unclear. Newer approaches like epigenome-wide association studies (EWAS) are at the helm to understand the disease etiology (Lunnon et al., 2014; de Jager et al., 2014). Such relatively new strategy has brought about evocative insights in the understanding of AD. Powerful techniques like the Illumina Human Methylation 450k BeadChip assay have paved the way to perform EWAS analysis of DNA methylation to investigate methylomic variations in the study of AD (Pidsley et al., 2013). This study, the first of its kind, revealed important AD related genes, the transcription of which, are controlled by DNA methylation (Lunnon et al., 2014; de Jager et al., 2014). DNA methylation is a chemical modification in which a methyl group is covalently attached to the 5′C of cytosine typically in a CpG island. Such heritable modifications are called epigenetic and are responsible for gene silencing.

The hypothesis behind our experiment comes from the fact that methylation influences the regulation of genetic elements in a variety of ways. DNA methylation in the transcription factor binding sites (TFBSs) is known to affect the regulatory mechanism of transcription factors (TFs) (Ehrlich and Ehrlich, 1993) which in turn has inhibitory effect on the transcription of the downstream miRNAs and genes. In certain cases methyl-CpG binding proteins can recruit histone deacetylases to facilitate histone modification in methylated DNA (Wade et al., 1999). This histone modification affects the binding of TFs. Again, the transcription start sites (TSSs) might also get blocked by the differential methylation, thereby causing a disease.

In light of the above, we aimed here to determine the significant differentially methylated regions of the brain that might bring about an anomalous expression of miRNAs leading to the cause of AD phenotype. To verify this, we obtain the differential methylated region (DMR) data from the study conducted by Lunnon et al., and CpG data from the UCSC genome browser. We then combine the two data sets to obtain the CpG islands that are differentially methylated in AD brains. The obtained CpGs with significant DMRs are then mapped to the 10 kb region upstream of miRNAs. The miRNAs with significant amount of DMRs in their promoter regions are finally put together to perform enrichment analysis for verifying the AD related biological processes and cellular components they are involved in. Further study of the feed forward loops (FFLs) between the TFs, gene targets, and the identified differentially methylated miRNAs reveals significant association with AD.

2. Related works

The anomalies in the expression and functionality of miRNAs have been shown to be related with a wide range of human diseases and this highlights their potential to be disease biomarkers (Chang and Mendell, 2007). With the first demonstration of the role of miRNAs in neuronal development in *C. elegans* (Busche et al., 2008), research on miRNA in the central nervous system (CNS) took a leap and several research groups identified a number of miRNAs that are cell-specific within the brains: such as the miRNAs -23, -26 and -29 are more abundant in astrocytes, while miRNAs-124 and -128 are predominant in neurons (Maes et al., 2009).The miRNAs play important functional roles in the maintenance of brain function such as synaptic transmission, regulation of spine structure of dendrites or even as mediators of synapse development (Schonrock et al., 2010). Dysregulation of miRNAs in the CNS thus leads to several neurological disorders and are the cause of neurodegenerative diseases like Alzheimer's disease and Parkinson's disease, neuropsychological diseases like schizophrenia or even autism spectrum disorders such as Fragile X syndrome and Rett syndrome (Wang et al., 2012).

Understanding the role of miRNAs in neurological disorders uses two basic approaches. One is by disrupting the miRNA biogenesis pathway and the other includes the silencing of a single miRNA to understand its specific role in the disease (Wang et al., 2012). A different way of studying the overall systematics of miRNAs in a particular disease phenotype is to use microarray technique and such implication in the study of AD pathology is proved successful. For example, a study conducted by Schipper et al., based on microarray data revealed several miRNAs that are overexpressed in blood mononuclear cells of sporadic AD patients (Schipper et al., 2007). Another study on differentially co-expressed miRNAs using microarray data conducted by Bhattacharyya et al., revealed a large number of miRNAs in the white matter of the brain is responsible for early AD progression (Bhattacharyya and Bandyopadhyay, 2013). Such studies are conducted by checking miRNA levels using microRNA microarray (MMChip) followed by validation of the results by RT-PCR.

The new trend however, in understanding AD pathology is by performing meta-analysis of genome-wide association study (GWAS) in individuals with AD (Chang and Mendell, 2007), the study of which has exposed a large number of genes associated with the disorder. But, the underlying mechanism of AD pathology still remains unclear. Hence with the aim to fill up the void, scientists have turned towards epigenetic studies to understand the disease in a new light. Two independent studies published at the same time showcased the first epigenome-wide associated studies (EWAS) in Alzheimer's disease (Lunnon et al., 2014; De Jager et al., 2014). These studies brought into light that several genes that are present within differential methylation regions of the brain are responsible for AD etiology. Several researches over the past decade have brought into attention that while miRNAs control the regulation of their target mRNAs, they themselves are also under tight control. For example, miR-132 is regulated by the cAMP response element binding (CREB) protein pathway (Wayman et al., 2008). On the other hand, miR-125a that reversibly regulates mRNA PSD-95 is in turn regulated by gp1 mGluR signaling pathway (Muddashetty et al., 2011). It is thus evident that miRNAs are also under the regulation by mechanism similar to mRNA regulations such as splicing, heterochromatin formation and even epigenetic silencing like histone modification and DNA methylation. We, in our study investigate to understand if there is a significant correlation between DNA methylation and miRNA transcription in AD brain.

3. Results

We have studied the differential epigenetic patterns in the promoter of miRNAs to establish their involvement in the progression of AD. We expect that differentially methylated regions overlapping with the TFBSs or TSSs will affect the transcription of the miRNAs, thereby causing a disease in an abnormal condition. Therefore, to identify the miRNAs related to AD from their epigenetic signatures, precisely the differential methylation patterns, we have performed a set of successive bioinformatic analyses. These are detailed hereunder.