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Increased micro-RNA 29b in the aged brain correlates with the reduction of insulin-like growth factor-1 and fractalkine ligand

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ABSTRACT

Microglia develop an inflammatory phenotype during normal aging. The mechanism by which this occurs is not well understood, but might be related to impairments in several key immunoregulatory systems. Here we show that micro-RNA (miR)-29a and miR-29b, 2 immunoregulatory micro-RNAs, were increased in the brain of aged BALB/c mice compared with adults. Insulin-like growth factor-1 (IGF-1) and fractalkine ligand (CX_3CL1) are negative modulators of microglial activation and were identified as targets of miR-29a and miR-29b using luciferase assay and primary microglia transfection. Indeed, higher expression of miR-29b in the brain of aged mice was associated with reduced messenger RNA (mRNA) levels of IGF-1 and CX_3CL1. Parallel to these results in mice, miR-29a and miR-29b were also markedly increased in cortical brain tissue of older individuals (mean, 77 years) compared with middle-aged adults (mean, 45 years). Moreover, increased expression of miR-29b in human cortical tissue was negatively correlated with IGF-1 and CX_3CL1 expression. Collectively, these data indicate that an age-associated increase in miR-29 corresponded with the reduction of 2 important regulators of microglia, IGF-1 and CX_3CL1.

1. Introduction

An increase in the inflammatory potential of the brain is a normal consequence of aging. For example, inflammatory cytokines including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 are increased in the brain of aged mice (Godbout et al., 2005; Njie et al., 2012; Ye and Johnson, 1999). Microglia are resident innate immune cells of central nervous system (CNS) and contribute to the increased level of proinflammatory cytokine expression in the aged brain (Corona et al., 2012). In support of this notion, several studies indicate that microglia from aged rodents have a "primed" phenotype with increased expression of major histocompatibility complex (MHC) II (Frank et al., 2006; Henry et al., 2009). This is important because primed ($MHCII^+$) microglia produce exaggerated levels of IL-1β in the brain of aged mice after a peripheral immune challenge (Henry et al., 2009). Increased IL-1β production is associated with dendritic atrophy, acute cognitive impairment, and prolonged sickness and depressive-like complications (Chen et al., 2008; Godbout et al., 2005, 2008; Richwine et al., 2008). Though the cause of microglial priming with age is unclear, several studies indicate that a decrease in microglial regulatory systems might be involved. For instance, brain aging is associated with reduced expression of several mediators of microglial regulation including anti-inflammatory cytokines (e.g., IL-10, IL-4) (Maher et al., 2005; Ye and Johnson, 2001a), neuronal-derived proteins (CD200, fractalkine ligand [CX_3CL1]) (Jurgens and Johnson, 2012; Lyons et al., 2007; Wynne et al., 2010), and growth factors (insulin-like growth factor [IGF]-1, nerve growth factor) (Deak and Sonntag, 2012; Larkfors et al., 1987; Sonntag et al., 2005b).

In rodent models of aging, CX_3CL1 is an integral modulator of microglia activation that is decreased in the brain with age (Bachstetter et al., 2011; Deak and Sonntag, 2012; Lyons et al., 2009; Wynne et al., 2010). Within the brain, CX_3CL1 is a chemokine constitutively expressed by neurons that binds to the fractalkine receptor (CX_3CR1) on microglia (Harrison et al., 1998). Thus, CX_3CL1–CX_3CR1 binding creates a unique regulatory relationship between neurons and microglia (Cardona et al., 2008). Disruption of this CX_3CL1–CX_3CR1 signaling pathway by loss of either CX_3CL1 or

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CX_3CR1 allows for inflammatory-induced activation of microglia (Cardona et al., 2006; Lyons et al., 2009; Wynne et al., 2010). For example, an age-associated reduction in CX_3CL1 corresponds with an increased number of primed/$MHCII^+$ microglia (Bachstetter et al., 2011), increased IL-1β production in the hippocampus (Lyons et al., 2009), and increased reactivity of microglia to a secondary immune challenge (Wynne et al., 2010). This increased microglial activity has functional consequences of reduced learning and memory (Rogers et al., 2011), reduced neurogenesis (Bachstetter et al., 2011), and increased depressive-like behavior (Godbout et al., 2008). Moreover, central infusion of CX_3CL1 reduced the primed $MHCII^+$ microglia profile in the brain of aged rats (Bachstetter et al., 2011; Lyons et al., 2009). The idea that CX_3CL1 is important in modulating microglia responses within the brain is also supported by studies using mice deficient in the fractalkine receptor (CX_3CR1^{KO}). CX_3CR1^{KO} mice have a hyperreactive microglial response to an inflammatory challenge (Cardona et al., 2006) that results in amplified proinflammatory cytokine production, prolonged sickness behavior, and the development of depressive-like behavior (Corona et al., 2010). Therefore, CX_3CL1–CX_3CR1 interactions are critical in the modulation of microglial activation.

IGF-1 is another modulator of microglia activation that is reduced in aged brain. Classically, IGF-1 is a growth factor that increases neuroprotection (Sonntag et al., 2005a, 2005b), neurogenesis (Llorens-Martin et al., 2009), long-term potentiation (Maher et al., 2006; Sonntag et al., 2005b), and dendritic growth and complexity (Niblock et al., 2000). IGF-1, however, can also modulate immune function. For example, IGF-1 reduces inflammatory cytokine responses in the brain (O'Connor et al., 2008) and ameliorates lipopolysaccharide (LPS)-induced sickness behaviors (Dantzer et al., 1999) that are primarily driven by microglia-dependent production of IL-1β and TNF-α (Dantzer et al., 2008). Moreover, central injection of a viral vector that upregulated IGF-1 in a mouse model of amyotrophic lateral sclerosis reduced microglial secretion of TNF-α and nitric oxide (Dodge et al., 2008). Thus, age-related reduction in IGF-1 might also contribute to the enhanced inflammatory profile of microglia in the aged brain.

We hypothesize that the reduction of multiple microglial regulatory pathways with age, including CX_3CL1 and IGF-1, indicate that there is loss of a global regulator of gene and protein expression. One possibility is that micro-RNA (miRNA) regulation is altered in the aged brain. miRNAs are small (19–24 nucleotides in length) noncoding RNAs that reduce posttranscriptional gene expression by binding to complementary target regions on mRNA to inhibit translation or promote messenger RNA (mRNA) degradation (Ambros, 2004; Bartel, 2004). miRNAs provide global regulation of gene expression and influence inflammatory processes (Baltimore et al., 2008; O'Connell et al., 2010). Target prediction algorithms of CX_3CL1 and IGF-1 revealed that both of these genes are potentially regulated by the micro-RNA (miR)-29 cluster. We have previously shown that the miR-29a/b cluster is upregulated in immune cells during the course of chronic inflammation, including multiple sclerosis (MS) and the animal model of MS, experimental autoimmune encephalomyelitis (Smith et al., 2012). Moreover, miRNAs in the miR-29 cluster were increased in the liver and muscle in a rodent model of accelerated aging (Zmpste24-null mice) (Ugalde et al., 2011). Thus, increased expression of miR-29 in the more inflammatory aged brain might contribute to the progression of microglial dysregulation and hyperactivity.

The purpose of this study was to determine the degree to which immunomodulatory miRNAs were altered in the aged brain and investigate their potential influence on microglial regulatory systems. Here, we show that miR-29a and miR-29b were increased in the brain of aged mice and older humans. Moreover, this increase in miR-29a and miR-29b expression was associated with the downregulation of specific CNS targets involved in the modulation of microglial activation, IGF-1 and CX_3CL1. Indeed, increased expression of miR-29b in the brain of older humans significantly and negatively correlated with IGF-1 and CX_3CL1 expression. We interpret these results to suggest that age-associated increases in miR-29 in the brain suppress multiple factors, including IGF-1 and CX_3CL1, contributing to the development of an inflammatory profile of microglia in the aged brain.

2. Methods

2.1. Mice

Adult (2–3-month-old) male BALB/c mice were obtained from a breeding colony kept in barrier-reared conditions in a specific pathogen-free facility at the Ohio State University. Aged (18–20-month-old) male BALB/c mice were obtained from the National Institute on Aging specific pathogen-free colony (maintained at Charles River Laboratories, Inc). Aged mice were allowed 1 week to acclimate to the facility before experimentation. All mice were individually housed in polypropylene cages and maintained at 25 °C under a 12-hour light/dark cycle with ad libitum access to water and rodent chow. All procedures were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.2. Postmortem human brain tissue

Postmortem human brain tissue was obtained from the Harvard Brain Tissue Resource Center (Belmont, MA, supported by the PHS grant R24 MH068855) (10 samples) and the Human Brain and Spinal Fluid Resource Center (Veterans Affairs West Los Angeles Healthcare Center, Los Angeles, CA, which is sponsored by the National Institute of Neurological Disorders and Stroke/National Institute of Mental Health, National Multiple Sclerosis Society, and the Department of Veterans Affairs) (17 samples). The tissue consisted of 11 adult (14–55 years old with a mean age of 45 years old) and 16 aged (58–91 years old with a mean age of 73 years old) brain samples (Table 1). The differentiation between "adult" or "aged" brain tissue was 57 years (younger than 57 = adult; older than 57 = aged). Tissue sections were collected from the normal-appearing white matter or postcentral parietal area (Brodmann areas 3, 1, 2, 5) and all samples were immediately flash frozen in liquid nitrogen (−196 °C) after collection, shipped on dry ice, and stored at −80 °C until use. All brains were designated healthy and non-CNS diseased controls with the exception of 4 samples obtained from patients with epilepsy (3 adult, 1 aged).

2.3. Microglial isolation

An enriched population of microglia was isolated from whole brain homogenates of mice as previously described (Fenn et al., 2012; Wynne et al., 2010). In brief, brains were homogenized in phosphate buffered saline through a 70-μm nylon cell strainer. Resulting homogenates were centrifuged and cell pellets were resuspended in 70% isotonic Percoll. A discontinuous Percoll density gradient was layered, centrifuged, and enriched microglia were collected from the interphase between the 70% and 50% Percoll layers. Microglia were washed and resuspended in phosphate buffered saline. Each brain extraction yielded approximately 3×10^5 viable cells. We have previously characterized these cells as enriched (approximately 85%) microglia ($CD11b^+/CD45^{low}$) (Henry et al., 2009).