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Neurobiology of Aging xx (2012) xxx

NEUROBIOLOGY OF AGING

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ApoE ε4 is associated with eIF2α phosphorylation and impaired learning in young mice

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Received 21 February 2012; received in revised form 19 June 2012; accepted 24 June 2012

Abstract

Protein translation is regulated during both initiation and elongation phases to enable cells to accommodate for ever-changing environmental and internal states. Eukaryotic initiation factor-2 (eIF2)α, a major signaling pathway for responses to metabolic stress, controls translation initiation in various cells, including neurons, and affects cognitive functions. The main risk factor for sporadic Alzheimer's disease (SAD) is aging, and the main genetic risk factor reducing the age of SAD onset is the expression of apolipoprotein E (ApoE)4. We tested the hypothesis that both genetic and aging risk factors converge on the eIF2α pathway. Aged rodents showed increased eIF2α phosphorylation in the brain, indicating a shift in the rate of translation initiation with increasing age. Interestingly, mice overexpressing human ApoE4 already, at an early age, exhibited increased eIF2α phosphorylation together with mild impairment in cognitive tasks, compared with ApoE3 mice. These results suggest that the eIF2α pathway is linked to SAD, possibly via genetic as well as prolonged metabolic stress, and these findings position it as a new and important target for treatment of the currently incurable Alzheimer's disease.

Keywords: Translation regulation; eIF2α

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, and the 5th leading cause of death in people aged 65 and older, and only symptomatic treatment is practicable (Galimberti and Scarpini, 2010; Ringman and Cummings, 2006). AD can be divided into sporadic and familial forms, and less than 5% of AD cases are classed as familial AD; they have early onset (before 65 years of age), characterized by autosomal dominant inheritance. Mutations in 3 different genes have been identified as risk factors for familial AD: amyloid precursor protein, presenilin 1, and presenilin 2. More than 90% of AD cases are sporadic AD; they have late onset (older than 65 years of age), and show a clear correlation between age and disease onset (reviewed in Bertram and Tanzi, 2008; Tanzi and Bertram, 2005). Although recent studies have suggested additional genes that may be involved (Lambert et al., 2009), the most central factor known to be a correlative genetic risk factor for sporadic AD is the apolipoprotein E (ApoE)4 gene, located on chromosome 19. ApoE encodes a 299-amino acid, 34-kDa glycoprotein with greatest production in the liver, followed by the brain. In the brain it is mostly secreted by astrocytes and, to some extent, by microglia (Pitas et al., 1987; Uchihara et al., 1995). However, neurons can also produce ApoE under certain conditions (Xu et al., 1999, 2006). The 3 ApoE isoforms—ε2, ε3, and ε4—differ from one another at residues 112 and 158 in ways that affect their 3-dimensional structure. The ε4 allele has approximately 20% occupancy in general populations and more than 50% in patients with AD (Rall et al., 1982; Strittmatter and Roses, 1996).

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0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.06.020

ApoE plays roles in: transport of cholesterol and other lipids into the central nervous system (Gong et al., 2007); interaction and clearance of amyloid beta (Aβ) (Belinson and Michaelson, 2009; Deane et al., 2008; Kim et al., 2009); modulation of inflammatory responses to cellular damage in the brain; modulation of oxidative injury (Colton et al., 2002; Jofre-Monseny et al., 2008); and maintenance of synaptic integrity (Lauderback et al., 2001). In addition, ApoE4 may induce memory deficit (Belinson et al., 2008; Bour et al., 2008; Grootendorst et al., 2005; Hartman et al., 2001), however, the underlying mechanisms connecting ApoE4 to AD are poorly understood.

Given that aging is the leading risk factor for AD, a straightforward hypothesis might suggest that a continuous metabolic stress is a prominent factor in the initiation as well as the progression of the disease. Cells in general, and neurons in particular, respond to metabolic stress in various ways, and a prominent cellular program that responds to metabolic stress is translation regulation (Sonenberg, 2000). Initiation of protein translation is considered to be the "rate-limiting" step; therefore it forms a major target for translational control following various forms of cellular stress (Holcik and Sonenberg, 2005). The initiation phase in protein translation involves several steps that utilize many factors. First, a ternary complex, termed the "pre-initiating complex," is formed, to enable the 40S ribosome to bind to the messenger RNA, thus leading to formation of the 80S ribosomal complex (Sonenberg, 2000). The ternary complex consists of eukaryotic initiation factor-2 (eIF2) bound to guanosine-5′-triphosphate (GTP) and methionyl transfer-RNA (Met tRNA). Phosphorylation of ser-51 in the α-subunit of eIF2 maintains binding of eIF2 to guanosine-5′-diphosphate (GDP), and consequently precludes its recruitment to a new ternary complex, and thereby inhibits general initiation of translation (Dever, 2002). Several cellular stressors induce activation of 4 different kinases that are responsible for phosphorylation of eIF2α (Sonenberg, 2000). These kinases are double-stranded RNA-activated protein kinase (PKR); general control nonderepressible-2 (GCN2); PKR-endoplasmic reticulum-related kinase; and hemin-regulated inhibitor kinase. In addition 2 identified genes, growth arrest and DNA-damage-inducible protein-34 and constitutive repressor of eIF2α phosphorylation encode the substrate-targeting subunits of 2 phosphatase complexes that independently dephosphorylate eIF2α (reviewed in Ron and Walter, 2007). Several signaling pathways regulate various factors in the machinery of translation initiation, and are considered to be hub molecules. These include the mammalian target of rapamycin (mTOR) and the extracellular signal-regulated kinase (ERK) signaling cascades (Gelinas et al., 2007; Wang and Proud, 2007).

Because postacquisition memory stabilization in diverse brain structures and species is protein-synthesis dependent, during the recent decade we and others analyzed the role of translation regulation in memory consolidation. Indeed, different factors in the translational pathway were found to be involved in learning and in synaptic plasticity processes (Belelovsky et al., 2005, 2009; Costa-Mattioli and Sonenberg, 2008; Richter and Klann, 2009). Interestingly, some genetic or pharmacological interventions in translation regulation may either interfere with learning or enhance it, depending on the direction of manipulation (Costa-Mattioli and Sonenberg, 2008). In particular, the phosphorylation level of Ser 51 on the eIF2α, bidirectionally regulates learning and synaptic plasticity: increased phosphorylation attenuated consolidation of memory and synaptic plasticity, and decreased levels enhanced them (Costa-Mattioli et al., 2007). Although most intensive research into the progression and etiology of AD focuses mainly on Aβ toxicity, several lines of research indicated that PKR activation and phosphorylation of eIF2α are involved in AD pathogenesis (Bando et al., 2005; Chang et al., 2002; Page et al., 2006; Peel and Bredesen, 2003), and that so is the mTOR signaling pathway (Pei and Hugon, 2008).

One would assume that aging, or physiological insults accumulated over the course of a lifetime, together with genetic predisposition, would have a major impact on the probability to develop AD. In order to test this general hypothesis with respect to specific genes and molecules, we analyzed the relationship between ApoE4, the major known risk factor for AD, and the eIF2 pathway, a well documented cellular stress indicator, in the brain. Explicitly, we compared levels of eIF2α phosphorylation in human-ApoE4 targeted-replacement mice born with 2 alleles of the ε4 with those in ApoE3 counterparts, at several different ages. Following the findings that ApoE4 mice already exhibited clear abnormalities in the eIF2 pathway at an early age, we tested ApoE4 mice and ApoE3 controls in hippocampus-dependent and -independent tasks, and observed clear differences between the 2.

2. Methods

2.1. Animals

Humanized knockin ApoE3 (B6.129P2-ApoE$^{tm2\ (ApoE*3)\ Mae}$ N8) and ApoE4 (B6.129P2-*ApoE*$^{tm3\ (ApoE*4)\ Mae}$ N8) homozygous male mice were supplied by Taconic (Hudson, NY, USA). Sprague–Dawley male rats were supplied by Harlan (Jerusalem, Israel). All cages were placed in a light- and temperature-controlled room, and behavioral tests were conducted during daylight hours. All animals were handled in accordance with the University of Haifa regulations and the National Institutes of Health Guidelines (NIH publication number 8023), and maintained in a pathogen-free environment.

2.2. Tissue preparation

After decapitation, the cortex and hippocampus were bilaterally dissected. Samples were homogenized in a glass and Teflon tissue homogenizer in 0.3 mL (for cortex) and