Impaired Eukaryotic Elongation Factor 1A Expression in Alzheimer’s Disease

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Key Words
Protein synthesis · Elongation factors · Eukaryotic elongation factor 1A · Mammalian target of rapamycin · Alzheimer’s disease

Abstract
Background/Aims: Recent studies have indicated a link between the impaired capacity of de novo protein synthesis and neurodegenerative diseases including Alzheimer’s disease (AD). Moreover, it has been established that eukaryotic elongation factor 1A (eEF1A) plays a critical role in maintaining long-term synaptic plasticity, a cellular model for learning and memory. The aim of the present study is to determine whether brain eEF1A protein levels are dysregulated in brain tissue from AD patients compared with controls. Methods: Postmortem human brain samples collected from patients clinically diagnosed as AD, and from age-matched healthy controls, were utilized for this study. Both Western blot and immunohistochemistry approaches were utilized to investigate the potential alteration of eEF1A protein levels by using a specific antibody. Results: Our data demonstrate that eEF1A expression is reduced in AD patients in the hippocampus, but not in the cerebellum or midfrontal gyrus. Furthermore, immunohistochemical experiments reveal that neuronal eEF1A reduction in the AD hippocampus is localized to the CA1 and dentate gyrus, but not to the CA3.

Conclusion: Dysregulation of eEF1A and its associated signaling pathways might represent novel molecular mechanisms underlying AD pathogenesis. Further investigation is necessary to determine whether eEF1A is a viable therapeutic target for AD and other cognitive syndromes.

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Introduction

A better understanding of the molecular mechanisms underlying the pathophysiology of Alzheimer’s disease (AD) could help identify novel diagnostic and prognostic biomarkers and potential therapeutic targets. Recent studies have suggested a link between disruption of protein synthesis homeostasis and neurological diseases including prion disease and AD [1, 2]. Mounting evidence has identified impairments of synaptic efficacy as an early and enduring key event in the process of AD-associated cognitive decline [3, 4], but understanding the mechanisms underlying this pathophysiology has been elusive. One possible mechanism may involve the eukaryotic mRNA translational factor eEF1A and its associated signaling pathways, which play important roles in maintaining long-lasting synaptic plasticity such as long-term potentiation, which is well known as a cellular model for learning and memory [5–7].
As one of the most abundant translational factors, eEF1A is a GTP-binding protein and responsible for the delivery of all aminoacylated transfer RNAs to the ribosome, and thus the ‘elongation’ of the polypeptide chain [8, 9]. Of interest, along with several other components of the mRNA translational machinery, the synthesis of eEF1A is known to be controlled by the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway [10]. Dysregulation of mTORC1 signaling has been implicated in AD, albeit varying results have been reported [11, 12]. Taken together, we hypothesize that eEF1A dysregulation may represent a novel molecular mechanism underlying AD pathophysiology. As the very first step to test our hypothesis, here we investigated eEF1A protein levels in different brain regions in postmortem human AD samples and compared them with age-matched controls. Our findings help lay the foundations for the future study of eEF1A-related therapeutics or diagnostics for AD, and potentially other age-related neurodegenerative processes that cause dementia.

**Methods**

**Subjects and Tissues**
De-identified postmortem human brain samples were obtained from the University of Washington (UW) Neuropathology Core in accordance with the UW and Wake Forest University School of Medicine Institutional Review Boards. Studies were performed using tissue from deceased patients in the UW Alzheimer’s Disease Research Center with clinically diagnosed (and neuropathologically confirmed) AD (n = 5) as well as age-matched controls with low levels of AD neuropathology (n = 5). Samples used in this study were from decedents who underwent a rapid autopsy shortly after death in which one half of the brain was dissected fresh and samples from diverse brain regions were flash frozen in liquid nitrogen. Nonfrozen portions of the dissected hemisphere, and the entire contralateral hemisphere, were fixed for approximately 2 weeks in 10% neutral buffered formalin and then underwent research quality diagnostic neuropathological examination, where at least 22 routine samples are collected and processed into formalin-fixed, paraffin-embedded tissue blocks. Clinical and neuropathological diagnoses were based on neurocognitive testing and postmortem Braak neurofibrillary tangle staging and Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) scores, respectively. Patient characteristics are shown in table 1. The mean age of death is 89.9 years. The postmortem interval ranged between 3 and 10 h with a mean of 5.63 h.

**Western Blot**
Frozen tissue samples received from the UW Neuropathology Core were kept on dry ice and sonicated in lysis buffer using a motorized Thermo Scientific (Waltham, Mass., USA) homogenizer. Lysis buffer was composed of T-Pe® Tissue Extraction Reagent (Thermo Scientific) with Hal™ Phosphatase Inhibitor Cocktail (Thermo Scientific) and Halt™ Protease Inhibitor Cocktail (Thermo Scientific). Homogenates were then centrifuged at 4°C for 10 min at 15,000 rpm. The supernatant was collected for protein quantification using the Pierce™ BCA Protein Assay Kit (Thermo Scientific). Samples containing 20 μg protein were loaded into precast Mini-Protean® TGX gels (4–10%; Bio-Rad, Hercules, Calif., USA) and resolved by standard gel electrophoresis. Protein was then transferred electrophoretically onto nitrocellulose membranes (0.2 μm; Bio-Rad) at room temperature. Membranes were blocked for 1 h in blocking buffer composed of 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20. All primary and secondary antibodies were diluted in blocking buffer. Blots were probed with primary antibodies for eEF1A (1:5,000; EMD Millipore, Billerica, Mass., USA) and GAPDH (1:10,000, Cell Signaling, Danvers, Mass., USA). Protein bands were visualized using chemiluminescence (Clarity™ ECL; Bio-Rad) and the Bio-Rad ChemiDoc™ MP Imaging System. Densitometric analysis was performed using ImageLab™ software (Bio-Rad). All data for eEF1A were normalized to GAPDH. Additionally, membranes were also probed for β-actin (1:10,000, Sigma Aldrich, St. Louis, Mo., USA) as another loading control (data not shown).

**Immunohistochemistry**
Tissue sections used for immunohistochemistry were prepared by the UW Neuropathology Core from formalin-fixed, paraffin-embedded tissue blocks described above. Sections were cut at 5 μm thickness, mounted on positively charged slides and baked for 30 min at 60°C. Sections were deparaffinized in xylene and rehydrated through a graded alcohol series. Antigen retrieval utilized citrate buffer (pH 6.0) in a standard 15-min microwave procedure. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 25 min. Slides were incubated in a humidified chamber in primary antibody for eEF1A (mouse; 1:1,000; EMD Millipore) overnight at 4°C or amyloid-β (6E10, mouse; 1:5,000; BioLegend, San Diego, Calif., USA) for 1 h at room temperature. Sections were then incubated in biotinylated α-mouse secondary antibody (1:200; Vector Labs, Burlingame, Calif., USA) for 30 min at room temperature followed by Vectastain® Elite ABC reagent (Vector Labs) for another 30 min. Primary and secondary antibodies as well as ABC re-

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**Table 1. Patient demographics**

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Age, years</th>
<th>Gender</th>
<th>PMI, h</th>
<th>Braak stage</th>
<th>CERAD score</th>
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<td>6</td>
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<td>2</td>
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<tr>
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<td>91</td>
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<td>5</td>
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<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>No dementia</td>
<td>92</td>
<td>F</td>
<td>6</td>
<td>III</td>
<td>Sparse</td>
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<tr>
<td>5</td>
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<td>97</td>
<td>F</td>
<td>10</td>
<td>III</td>
<td>Sparse</td>
</tr>
<tr>
<td>6</td>
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<td>82</td>
<td>F</td>
<td>8</td>
<td>VI</td>
<td>Frequent</td>
</tr>
<tr>
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<td>M</td>
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</tr>
<tr>
<td>8</td>
<td>AD</td>
<td>91</td>
<td>M</td>
<td>7</td>
<td>V</td>
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<td>IV</td>
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</tr>
</tbody>
</table>

PMI = Postmortem interval; CERAD = Consortium to Establish a Registry for Alzheimer’s Disease.

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agent were diluted in 1% BSA/PBS. Diaminobenzidine was diluted in Tris buffer (pH 7.7) and 3% hydrogen peroxidase in a working diaminobenzidine solution. Sections were developed in diaminobenzidine for 10 min in a 42°C water bath. Slides were counterstained using Mayer’s hematoxylin and blued with 0.2% lithium carbonate. In between each step of immunohistochemistry, sections were rinsed using distilled water or PBS (pH 7.4). Negative controls were incubated in 1% BSA with no primary antibody. Sections were dehydrated in an alcohol series and cleared with xylene, cover-slipped and dried overnight. Imaging was performed using bright-field microscopy on a Zeiss Axioplan 2 Epifluorescent Microscope (Oberkochen, Germany) using Zen 2012 Imaging Software (Zeiss).

**Data Analysis**

Data analysis was conducted using Prism 6 (Graphpad Software, San Diego, Calif., USA). All data sets were subjected to the D’Agostino-Pearson normality test and determined to have a normal Gaussian distribution. Data are presented as group means with SEM, with p < 0.05 considered significantly different from controls. Group differences were assessed using the unpaired independent t test.

**Results**

**Levels of eEF1A Proteins Are Decreased in Hippocampi of Human AD Patients**

We set out to study whether eEF1A protein levels are altered in different brain regions by performing Western blot assays on protein extracts from postmortem human brain samples. Three brain regions were investigated including: hippocampus, cerebellum and midfrontal gyrus. As shown in figure 1, eEF1A levels in the hippocampus were significantly decreased in AD patients, compared to age-matched controls (p = 0.0087). In contrast, eEF1A expressions in either the cerebellum or midfrontal gyrus of AD samples were not distinguishable from those of control samples (p = 0.4458 and 0.9792, respectively).

**Hippocampal Neuronal eEF1A Levels Are Reduced in Human AD Patients**

To gain insight into the cellular distribution of AD-associated eEF1A dysregulation, we performed immunohistochemistry on formalin-fixed, paraffin-embedded sections. First, we confirmed the presence of AD pathology (amyloid plaques) in AD samples, and its absence in control tissues, using an antibody (6E10) for amyloid-β (fig. 2a). We then evaluated the pattern and intensity of eEF1A immunostains in adjacent sections, which revealed reduced eEF1A signals in CA1 pyramidal neurons and hilar neurons of the dentate gyrus in AD, compared to controls (fig. 2b, c). We did not observe any clear difference in eEF1A immunostaining in CA3 pyramidal neurons (fig. 2d), suggesting region- or neuron-specific regulation of eEF1A expression in AD. The results were consistent with the aforementioned findings from biochemical experiments and indicated impaired eEF1A expression and thus general protein synthesis in AD.

**Discussion**

By all measures AD is a significant public health issue and will grow in severity with population aging and is thus an impending health care disaster [13]. Unfortu-
nately, there is still no effective intervention available to slow progression or cure the disease, which makes it urgent to search for new therapeutic strategies based on fundamental mechanistic studies. Here we have demonstrated, in AD brains, significantly reduced levels of hippocampal eEF1A protein. It is interesting that we find differences specific to the hippocampus, which is critical for memory formation and considered to be involved relatively early in the progression of AD pathology. Further, the control tissues examined in this study were in Braak stages II or III, which indicates involvement of the CA1 and subiculum by neurofibrillary tangle pathology, while other CA regions (including CA3 and hilus of the dentate gyrus – CA4) are relatively uninvolved. This suggests that...
amyloid-β pathology, which is sparse or absent in control cases, but moderate to frequent in AD cases, may drive eEF1A differences more than tau pathology. Future experiments are needed to determine the association of eEF1A with specific AD pathological processes and the specificity of eEF1A changes to AD and other neurodegenerative processes.

As one of the most abundant mRNA translational factors, eEF1A plays a critical role in the elongation phase of de novo protein synthesis. Numerous studies have firmly established that de novo protein synthesis is essential for the maintenance of long-term synaptic plasticity and memory formation [14, 15]. In agreement, recent findings indicate a link between compromised de novo protein synthesis and AD-associated impairments of neuronal plasticity and cognitive function [2]. It is also worth mentioning that the regulation at the elongation phase is especially valuable when mRNA translation has already been started but remains dormant until needed, thus accelerating new protein production by bypassing the relatively slow initiation step [16].

Furthermore, mounting evidence indicates a relationship between AD pathophysiology and the mTORC1 signaling pathway [11, 12]. The mTORC1 controls protein synthesis in part through regulating synthesis of new translational apparatus via p70 S6 kinase. Particularity, the process leads to the translation of a specific class of mRNAs characterized by terminal oligopyrimidine in their 5′-untranslated regions. Proteins encoded by such transcripts include ribosomal proteins and elongation factors such as eEF1A [10]. The findings from the current study that eEF1A levels are downregulated in AD suggest an impaired mTORC1 signaling, which is consistent with our previous report [11].

Our long-term goal is to determine whether neuronal eEF1A dysregulation may represent a heretofore unknown molecular mechanism underlying AD pathogenesis. Experiments to correct impaired eEF1A dysregulation in an AD mouse model are ongoing to determine whether de novo protein synthesis can be restored and cognitive impairments can be rescued using this approach. These studies will provide clarity on whether eEF1A is a feasible therapeutic target for AD. Further studies of eEF1A in other age-related models of neurodegeneration are planned to determine how specific eEF1A alterations are to AD.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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