Neurobiology of Aging 34 (2013) 1412–1415

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Brief communication

Expression level of vascular endothelial growth factor in hippocampus is associated with cognitive impairment in patients with Alzheimer's disease

Huidong Tang [a,b], XiaoOu Mao [b], Lin Xie [b], David A. Greenberg [b], Kunlin Jin [b,c,*]

[a] Department of Neurology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[b] Buck Institute for Research on Aging, Novato, CA, USA
[c] Department of Pharmacology and Neuroscience, Institute for Aging and Alzheimer's Disease Research, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, USA

ARTICLE INFO

Article history:
Received 4 April 2012
Received in revised form 22 June 2012
Accepted 31 October 2012
Available online 24 November 2012

Keywords:
VEGF
Alzheimer's disease
Human
Brain
Expression

ABSTRACT

Although the enhanced expression of vascular endothelial growth factor (VEGF) in the brains of patients with Alzheimer's disease (AD) has been reported, the functional significance of VEGF level in the progression of AD is still unclear. We examined the VEGF expression in the hippocampus of patients with AD at different stages of progression by Western blot analysis, and found that the VEGF189 isoform ($VEGF_{189}$) was barely detectable in normal hippocampus, but significantly increased at the early stage of patients with AD. $VEGF_{189}$ was decreased with advancing stages of AD. Immunostaining shows that VEGF was significantly increased in the cells in the CA1, CA3, and dentate gyrus regions of hippocampus and layers III and V of entorhinal cortex of patients with AD, compared with normal brain. Confocal images show that VEGF was predominantly expressed in neurons and astrocytes in the hippocampus and entorhinal cortex of patients with AD. Our data suggest that VEGF level is associated with progressive loss of cognitive function in patients with AD.

1. Introduction

Several growth factors have been implicated in the pathogenesis of Alzheimer's disease (AD). One of them is vascular endothelial growth factor (VEGF). Genetic analysis indicates that single nucleotide polymorphisms within the VEGF gene promoter region confer greater risk for AD, vascular dementia, and frontotemporal lobal degeneration, but others could not repeat these findings (Chapuis et al., 2006). Abnormal expression level of VEGF is also observed in patients with AD. For example, enhanced VEGF immunoreactivity is observed in reactive astrocytes in the neocortex and large intraparenchymal vessels and capillaries of subjects with AD compared with elderly control subjects. AD is often accompanied by reactive astrogliosis and microglia activation, suggesting a role of VEGF in neurodegenerative processes associated with AD. In addition, VEGF is also heavily accumulated and colocalized with amyloid-beta (Aβ) plaques in the brains of patients with AD. Consistently, the serum VEGF levels in patients with AD are significantly lower than in control subjects, which could be because of the continuous deposition of VEGF in the amyloid plaques (Mateo et al., 2007). Decreased serum VEGF levels are associated with AD in a dose-dependent manner, the lower third of serum VEGF levels being associated with a 5-fold increased risk for AD when compared with the upper third (Mateo et al., 2007). However, other studies show that VEGF level is elevated in the cerebrospinal fluid and the peripheral blood of patients with AD (Corsi et al., 2011). VEGF in cerebrospinal fluid is correlated with the severity of cognitive impairment. Therefore, whether VEGF is increased in patients with AD remains a matter of debate, and the functional significance of VEGF level in the pathogenesis and progression of AD is still unclear. In the current study, we examined the VEGF expression levels in the hippocampus of patients with AD at different stages of progression by Western blot analysis, and found that the VEGF189 isoform ($VEGF_{189}$) level is associated with progressive loss of cognitive function in patients with AD. Immunostaining confirms increased VEGF expression in the neurons and astrocytes in the hippocampus and entorhinal cortex of AD brain.

2. Methods

2.1. Human brain tissue

Brain tissue was from the Harvard Brain Tissue Resource Center, and the Brain and Tissue Bank for Developmental Disorders at the

* Corresponding author at: Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX, USA. Tel.: +1 817 735 2579; fax: +1 817 735 4080.
E-mail addresses: kjin@buckinstitute.org, kunlin.jin@unthsc.edu (K. Jin).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.10.029

University of Maryland at Baltimore. Twenty-five postmortem brains were used: 14 from individuals with a clinical diagnosis of probable AD and 6 (control brains) from individuals without neurological disorders as shown in our previous publication (Jin et al., 2004). Research was conducted in compliance with the policies and principles contained in the Federal Policy for the Protection of Human Subjects.

2.2. Western blot analysis

Hippocampi were isolated from frozen brains and protein was isolated and Western blot analysis was performed as in our previous publication (Jin et al., 2004). Primary antibody was mouse monoclonal anti-VEGF (Santa Cruz Biotechnology; 1:1000) and secondary antibody was horseradish peroxidase-conjugated anti-mouse secondary antibody (Santa Cruz Biotechnology; 1:3000). The membranes were reprobed with mouse monoclonal anti-actin (Oncogene Science; 1:20,000). Differences in protein expression were quantified by a GS-710 calibrated imaging densitometer and Quantity One 4.2.1 software (Bio-Rad).

2.3. Immunohistochemistry

Immunohistochemistry and double immunostaining were performed as in our previous publication (Jin et al., 2004). The primary antibodies used, in addition to monoclonal anti-VEGF antibody, were rabbit polyclonal anti-VEGF (Chemicon; 1:250), mouse monoclonal anti-glial fibrillary acidic protein (GFAP) (Sigma; 1:500) and mouse monoclonal anti-neuronal nuclear antigen (NeuN) (Chemicon; 1:250). The secondary antibodies were Alexa Fluor 488-, 594-, or 647-conjugated donkey anti-mouse or anti-rabbit IgG (Molecular Probes, 1:200–500). Controls included omitting either the primary or secondary antibody or preabsorbing the primary antibody. The slide examiners were blinded to the source of the specimen (AD vs. control).

2.4. Statistical analysis

Quantitative results were expressed as the mean ± standard error of the mean. The statistical significance between means was evaluated using 1-way analysis of variance. $p < 0.05$ was considered statistically significant.

3. Results

To investigate endogenous level of VEGF in AD hippocampus, we first used protein from AD and control hippocampus to perform Western blot analysis with antibody against human VEGF. We found that protein at the predicted molecular sizes (26 kDa) for human $VEGF_{189}$ was weakly expressed in the normal hippocampus (Cressey et al., 2005). The expression of $VEGF_{189}$ was increased in hippocampus of AD patients (Fig. 1A), particularly in hippocampus of patients in the early stage of AD. There appeared to be a tendency for expression to decrease with increasing progressive loss of cognitive function and memory (Fig. 1B). In addition, we found that protein at the predicted molecular size (18 kDa) for human VEGF121 isoform ($VEGF_{121}$) was reduced in the AD hippocampi (Cressey et al., 2005). Actin expression was used as a control for protein loading.

Next, we asked whether Western blot data could be confirmed by immunohistochemistry. As shown in Fig. 2A and B, VEGF was expressed in cells in the CA1, CA3, and dentate gyrus regions of hippocampus and the layer III and V of entorhinal cortex of AD patient brains. However, VEGF was weakly expressed only in a few cells in normal hippocampus and entorhinal cortex (Fig. 2C). VEGF

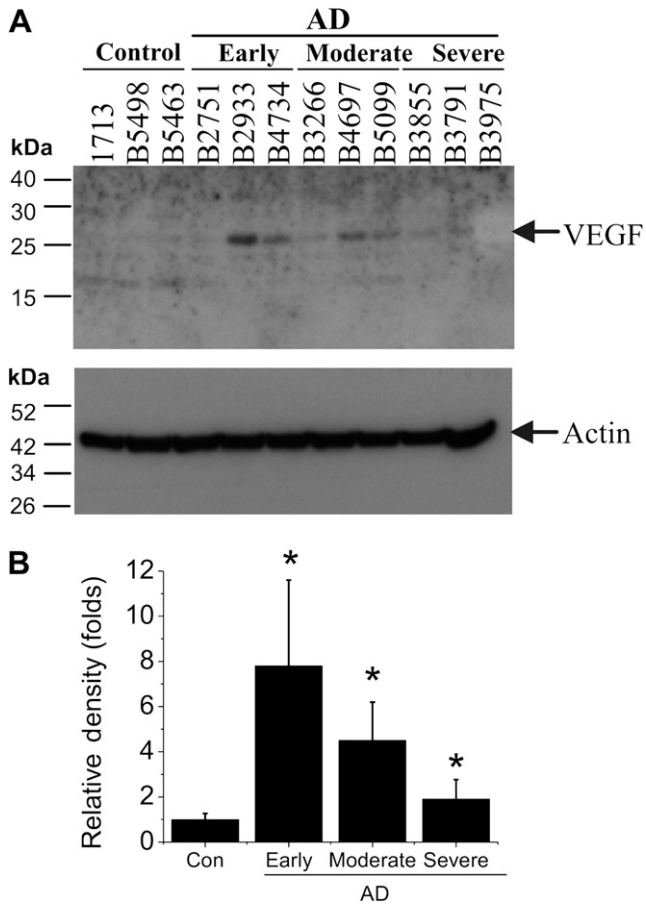

Fig. 1. Expression of vascular endothelial growth factor (VEGF) protein in Alzheimer's disease (AD) hippocampus. (A) Protein from control (Con) and AD hippocampus at the early, moderate, and severe stages of AD was used for Western blot analysis with antibody against human VEGF and actin was used as a control for consistency of protein loading. The arrow labeled VEGF indicates the predicted size for VEGF189 (26 kDa). The band seen at 18 kDa corresponds to the predicted size of VEGF121 (18 kDa). (B) VEGF189 band intensities were quantified by computer-assisted densitometry to give average values (fold increase over same-gel control). * $p < 0.05$.

protein was localized in the cytoplasm of cells in both regions. Double-labeled immunostaining shows that VEGF was predominantly expressed in neurons in the pyramidal layer of the hippocampus and entorhinal cortex of patients with AD (neuronal nuclear antigen-positive cells; Fig. 2D). In addition, VEGF was expressed in some astrocytes (GFAP-positive cells; Fig. 2E) in the entorhinal cortex of patients with AD (Fig. 2B).

4. Discussion

In this study, we found that VEGF ($VEGF_{189}$) protein is increased in hippocampus of patients with AD, and expression level of $VEGF_{189}$ is related to stages of cognitive impairment of AD. We also found that VEGF is expressed not only in neuronal cells, but also in astrocytes of hippocampus and entorhinal cortex of patients with AD.

Growing evidence has shown that vascular diseases and vascular risk factors are associated with AD, and some epidemiological studies suggest that cardiovascular drugs with an anti-angiogenic effect have significant clinical effects on Aβ deposition in AD and might influence the outcome of AD patients (Isingrini et al., 2009). VEGF appears to play a critical role in the neurobiology of Aβ deposition in the brains of patients with AD, because Aβ inhibits not only VEGF-induced migration of endothelial cells, but also