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Expression Level of Vascular Endothelial Growth Factor in Hippocampus is Associated with Cognitive Impairment in Patients with Alzheimer's Disease

Huidong Tang^{1,2}, XiaoOu Mao², Lin Xie², David A. Greenberg², and Kunlin Jin^{1,2,3}

¹Department of Neurology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

²Buck Institute for Research on Aging, Novato, California 94945, USA

³Department of Pharmacology and Neuroscience, Institute for Aging and Alzheimer's Disease Research, University of North Texas Health Science Center at Fort Worth, Texas 76107, USA

Abstract

Although the enhanced expression of 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, and found that VEGF (VEGF₁₈₉) was barely detectable in normal hippocampus, but significantly increased at the early stage of patients with AD. VEGF₁₈₉ 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 the layer III and V of entorhinal cortex of patient with AD, compared to normal brain. Confocal images show that VEGF was predominantly expressed in neurons and astrocyte 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.

Keywords

VEGF; Alzheimer's disease; Human; brain; expression

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 the single nucleotide polymorphisms (SNPs) within VEGF gene promoter region confer greater risk for AD, vascular dementia and frontotemporal lobar degeneration, while others could not repeat these findings [2]. Abnormal expression level of VEGF is also observed in patients with AD. For example, enhanced VEGF immunoreactivity is observed in reactive

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Corresponding author: Kunlin Jin, M.D., Ph.D., Professor, Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX, USA, Tel: 817-735-2579, Fax: 817-73500408, kunlin.jin@unthsc.edu.

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astrocytes in the neocortex and large intraparenchymal vessels and capillary of subjects with AD compared to elderly controls. 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 co-localized with A β plaques in the brains of patients with AD. Consistently, the serum VEGF levels in patients with AD is significantly lower than that of the controls, which could be due to the continuous deposition of VEGF in the amyloid plaques [10]. Decreased serum VEGF levels are associated with AD in a dose-dependent manner, the lower third of serum VEGF levels being associated with a fivefold increased risk for AD when compared with the upper third [10]. However, other studies show that VEGF level is elevated in the cerebrospinal fluid (CSF) and the peripheral blood of patients with AD [3]. VEGF in CSF 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, and found that VEGF (VEGF₁₈₉) 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.

MATERIAL AND METHODS

Human Brain Tissue

Brain tissue was from the Harvard Brain Tissue Resource Center, and the Brain and Tissue Bank for Developmental Disorders at the University of Maryland at Baltimore. Twenty-five postmortem brains were used: 14 from individuals with a clinical diagnosis of probable AD and 6 (controls) from individuals without neurological disorders as shown in our previous publication [8]. Research was conducted in compliance with the policies and principles contained in the Federal Policy for the Protection of Human Subjects.

Western Blotting

Hippocampi were isolated from frozen brains and protein was isolated and Western blot was performed as our previous publication [8]. 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; 1:3,000). The membranes were reprobed with mouse monoclonal anti-actin (Oncogene Science; 1:20,000). Differences in protein expression were quantified by using a GS-710 calibrated imaging densitometer and QUANTITY ONE software (Bio-Rad).

Immunohistochemistry

Immunohistochemistry and double immunostaining were performed as our previous publication [8]. The primary antibodies used, in addition to VEGF antibody, were mouse monoclonal anti-GFAP antibody (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).

Statistical analysis

Quantitative results were expressed as the mean $\pm$ SEM. The statistical significance between means was evaluated using one-way analysis of variance (ANOVA). $P < 0.05$ was considered as statistically significant.

RESULTS

To investigate endogenous level of VEGF in AD hippocampus, we first used protein from AD and control hippocampus to perform Western blots with antibody against human VEGF. We found that protein at the predicted molecular sizes (26kDa) for human VEGF₁₈₉ was weakly expressed in the normal hippocampus [4]. The expression of VEGF₁₈₉ was increased in hippocampus of AD patients (Figure 1A), particularly in hippocampus in the early stage of AD patients. There appeared to be a tendency for expression to decrease with increasing progressive loss of cognitive function and memory (Figure 1B). In addition, we also found that protein at the predicted molecular sizes (18kDa) for human VEGF₁₂₁ was reduced in the AD hippocampi [4]. 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 Figure 2A and B, VEGF was expressed in cells in the CA1, CA3 and dentate gyrus (DG) 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 of cells in normal hippocampus and entorhinal cortex (Figure 2C). VEGF 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 patient with AD (NeuN-positive cells; Figure 2D). In addition, VEGF was also expressed in some astrocytes (GFAP-positive cells; Figure 2E) in the entorhinal cortex of patient with AD (Figure 2B), suggesting the neuroprotective role of VEGF in human AD.

DISCUSSION

In this study, we found that VEGF (VEGF₁₈₉) protein is increased in hippocampus of patient with AD, and expression level of VEGF₁₈₉ is relative with stages of cognitive impairment of AD. We also found that VEGF are 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, as epidemiological studies show that cardiovascular drugs with an anti-angiogenic effect have significant clinical effects on A β deposition in AD and can influence the outcome of AD patients [7]. VEGF appears to play a critical role in the neurobiology of A β deposition in the brains of patients with AD, as A β inhibits not only VEGF-induced migration of endothelial cells, but also VEGF-induced permeability of an in vitro model of the blood brain barrier (BBB), which dysfunction may contribute to the pathogenesis of some AD lesions [11]. In addition, VEGF also exerts direct effects on neurons. We found that VEGF expression is also increased in neurons and astrocytes in the hippocampus and entorhinal cortex of AD brain, which are highly vulnerable regions in AD [6]. Our data suggest that VEGF may play a pivotal role in neural protection in AD as well, and lack of this activity may involve the development of AD. The fact that VEGF is increased at the early stage, but decreased with advancing stage of AD, could indicate the mechanism of self-repair in the early stage of the disease. Continuous deposition of VEGF in the amyloid plaques most likely results in deficiency of available VEGF in AD. In addition, differential expression levels of VEGF in different stages of progression may partially explain why

some studies reported that no difference in serum VEGF levels was detected in AD patients[5], but other studies state VEGF level in serum is altered [10].

Our previous study shows that VEGF can also stimulate neurogenesis in adult brain [9,12]. A recent study shows that overexpression of VEGF in adult rats resulted in increased neurogenesis associated with improved cognition, and mutant KDR inhibits basal neurogenesis and impaired learning [1], suggesting that VEGF facilitates memory and learning through stimulating neurogenesis. Memory loss is the most noticeable deficit in patients with AD. These findings suggest that involvement of VEGF in the cognitive impairment and pathogenesis of AD may associate with VEGF-mediated neuroprotection and neurogenesis besides angiogenesis.

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Figure 1. Expression of VEGF protein in AD hippocampus

(A) Protein from control (Con) and AD hippocampus at the early, moderate, and severe stages of AD was used for Western blotting with antibody against human VEGF and actin was used as a control for consistency of protein loading. (B) Band intensities were quantified by computer-assisted densitometry to give average values (fold increase over same-gel control). * $P < 0.05$.

Figure 2. The phenotype of VEGF-positive cells in the brain of patients with AD

(A) Immunohistochemical evidence for VEGF expression in neurons in the CA1, CA3 and DG regions of hippocampus of AD brains. Left panel: low magnification; right panel: high magnification. (B) VEG was expressed in the cells in the layer III and V of entorhinal cortex of AD brains. Left panel: low magnification; right panel: high magnification. (C) VEGF-positive cells were barely observed in the normal hippocampus (up panel) and entorhinal cortex (bottom panel). (D) Confocal imaging shows that VEGF-positive cells (green) were colocalized with NeuN-positive cells (red) from hippocampus and entorhinal cortex of AD brains. Nuclei were counterstained with DAPI (blue). (E) VEGF (green) was expressed in GFAP-positive cells (red) from entorhinal cortex of AD brains. Nuclei were counterstained with DAPI (blue). Hippo: hippocampus; Ctx: cortex.