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Research Article

AKT ACTIVATION AND GSK-3 β INACTIVATION IN THE HIPPOCAMPUS ARE ACCELERATED BY DELETING IL-33

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ABSTRACT

The present study investigated the role of interleukin-33 (IL-33), a proinflammatory cytokine, in the activities of Akt and glycogen synthase kinase 3 β (GSK-3 β). GSK-3 β phosphorylates tau protein, causing neurofibrillary tangles (NFT) in the brain of Alzheimer's disease (AD). The IL-33 protein levels in the hippocampus of 5xFAD mice, an animal model of AD, were significantly higher than the levels for wild-type control mice. Ser473 phosphorylation of Akt and Ser9 phosphorylation of GSK-3 β were significantly enhanced in the hippocampus of IL-33-deficient mice as compared with those for wild-type control mice. This indicates that Akt is more activated and GSK-3 β is more inactivated by deleting IL-33; i.e., IL-33 inactivates Akt and activates GSK-3 β , thereby stimulating tau phosphorylation and NFT formation. Overall, these results raise the possibility that IL-33 may be a factor to accelerate tau phosphorylation in the AD brain.

Key Words: IL-33-deficient mouse, Akt, GSK-3 β , 5xFAD mouse, Alzheimer's disease.

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INTRODUCTION

Alzheimer's disease (AD) is characterized by deposition of amyloid plaques consisting of amyloid β peptide and neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau protein in the brain. Glycogen synthase kinase 3 β (GSK-3 β), which is inactivated by being phosphorylated at Ser9 and activated by being phosphorylated at Tyr216, is the major factor to

phosphorylate tau (Jeganathan S *et al.*, 2008). Akt, which is activated by being phosphorylated at Thr308 and Ser473 (Takashima A, 2006), inactivates GSK-3 β by phosphorylating at Ser 9.

Interleukin-33 (IL-33), a member of the IL-1 family, is recognized as a proinflammatory cytokine as well as a nuclear factor (Han P *et al.*, 2011; Liew FY *et al.*, 2010). IL-33 activates its heterodimeric receptor complex ST2 and interleukin-1 receptor accessory protein (IL-1RAcP) linked to myeloid differentiation factor 88 (MyD88) (Nakae S *et al.*, 2013). Subsequently, MyD88 is involved in the activation of a wide variety of signaling cascades, that include extracellular signal-regulated kinase (ERK), activator protein 1 (AP-1), c-Jun-N-terminal kinase (JNK) along an IL-1 receptor-associated kinase (IRAK)/tumor necrosis factor (TNF) receptor associated factor 6 (TRAF6) axis, nuclear factor- κ B (NF- κ B) along an IRAK/TRAF6/I κ B kinase (IKK) axis, and endothelial NO synthase (eNOS) along an IRAK/TRAF6/phosphoinositide-3-kinase (PI3K)/Akt axis (Choi YS *et al.*, 2009; Nakae S *et al.*, 2013).

Accumulating evidence has pointed to the role of IL-33 in the pathogenesis of AD (Xiong Z *et al.*,

2014). The detail, however, is not fully understood. The present study investigated the effect of IL-33 on Akt and GSK-3 β . The results show that Akt activation and GSK-3 β inactivation in the hippocampus are promoted by deleting IL-33.

MATERIALS AND METHODS

Animal care

All procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

5xFAD mice (Tg6799)

5xFAD mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained by crossing heterozygous transgenic mice with B6/SJL F1 breeders. Non-transgenic wild-type littermate mice were used as controls.

IL-33-deficient mice

IL-33^{-/-} Balb/c mice were kindly provided by Prof. T. Yoshimoto (Hyogo College of Medicine, Nishinomiya, Japan)(Yasuda *et al.*, 2012). Respective littermate wild-type mice were used as controls.

Quantification of IL-33 protein and mRNA

The IL-33 mRNA was quantified in the real-time reverse transcription-polymerase chain reaction (RT-PCR) and the expression level was normalized by that of the GAPDH mRNA. IL-33 protein was assayed using a mouse IL-33 ELISA kit (Abcam, Cambridge, UK) and the expression level was normalized by tissue protein weight.

Western blotting

Hippocampal slices from mice (400 μ m) were lysed and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Blotting membranes were reacted with antibodies against Akt (Cell Signaling, Beverly, MA, USA), phospho-Thr308-Akt (pT308)(Cell Signaling), phospho-Ser473-Akt (pS473)(Cell Signaling), GSK-3 β (Cell Signaling), phospho-Ser9-GSK-3 β (pS9)(Cell Signaling), and phospho-Tyr216-GSK-3 β (pY216)(BD Biosciences, San Jose, CA, USA). After washing, membranes were reacted with a horseradish peroxidase-conjugated goat anti-mouse IgG or goat anti-rabbit IgG antibody (MP Biomedicals, Santa Ana, CA, USA). Immunoreactivity was detected with an ECL kit (Invitrogen, Carlsbad, CA, USA) and visualized using a chemiluminescence detection system (GE Healthcare, Piscataway, NJ, USA).

Statistical analysis

Statistical analysis was carried out using unpaired *t*-test.

RESULTS AND DISCUSSION

5xFAD mice with five familial forms of AD (FAD) mutations co-express the 695-amino acid isoform of the human amyloid precursor protein (APP) (APP695) carrying the Swedish/London/Florida mutations and human presenilin 1 (PS1) carrying the M146L/L286V mutations (Oakley H *et al.*, 2006). My first attempt was to understand the expression levels of the IL33 mRNA and protein in the hippocampus of 5xFAD mice. The IL-33 mRNA level for 5xFAD mice was significantly lower than that for wild-type control mice (Figure 1A). In contrast, the IL-33 protein level for 5xFAD mice was significantly higher than that for wild-type control mice (Figure 1B). This suggests that in spite of downregulation of the transcription, the IL-33 translation is upregulated in the AD brain.

When hyperphosphorylated, tau is missorted into the somatodendritic compartment and aggregates into NFT (Braak H and Braak E, 1991). GSK-3 β , a serine/threonine protein kinase, is recognized to phosphorylate tau. GSK-3 β is inactivated and activated through its phosphorylation at Ser9 and Tyr216, respectively (Jeganathan S *et al.*, 2008). Akt is also a serine/threonine protein kinase. Akt is activated through its phosphorylation at Thr308 and Ser473 along a receptor tyrosine kinase (RTK)/insulin receptor substrate 1 (IRS-1)/PI3K/3-phosphoinositide-dependent protein kinase 1 (PDK1)/Akt axis (Takashima A, 2006). Akt phosphorylates and inactivates GSK-3 β .

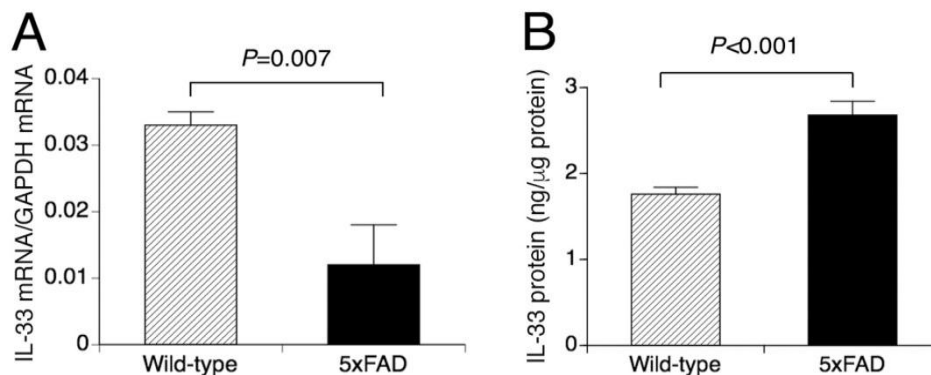
In the present study, Ser473 phosphorylation of Akt was significantly enhanced in the hippocampus of IL-33-deficient mice as compared with that for wild-type control mice, but there was significant difference in the Thr308 phosphorylation (Figure 2). This indicates that Akt is activated under the condition of IL-33 deficit. Ser9 phosphorylation of GSK-3 β was significantly enhanced in the hippocampus of IL-33-deficient mice as compared with that for wild-type control mice, although Tyr216 phosphorylation was not affected (Figure 3). This indicates that GSK-3 β is inactivated under the condition of IL-33 deficit. Taken together, these results raise the possibility that Akt activation and GSK-3 β inactivation are accelerated by deleting IL-33; in other words, IL-33 inactivates Akt, thereby activating GSK-3 β , to stimulate tau phosphorylation (Figure 4). IL-33, therefore, might worsen tauopathy in AD. IL-33, however, is shown to activate Akt through an (ST2/IL-1RAcP)/MyD88/TRAF6/PI3K pathway (Choi YS *et al.*, 2009) (Figure 4). The results of the present study may represent a pathway for IL-33-induced suppression of Akt (Figure 4).

In the preliminary study, Schaffer collateral/CA1 long-term potentiation (LTP) in the hippocampal slices from IL-33-deficient mice was significantly suppressed as compared with the LTP for wild-type control mice, which is reversed by adding IL-

33 (unpublished data). In the water maze test, the acquisition latency for IL-33-deficient mice was significantly prolonged as compared with the latency for wild-type control mice (unpublished data). These results indicate that IL-33 is required for LTP and spatial learning. If so, IL-33 should decrease in the brain of AD with cognitive impairment. This, however, is not the case here; expression of IL-33 protein was increased in the hippocampus of 5xFAD mice. In support of this finding, IL-33-positive cells increase in the AD brain

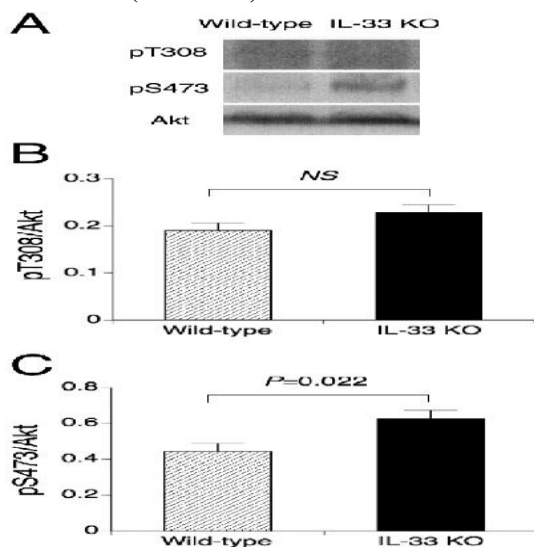
and amyloid β_{1-42} upregulates expression of IL-33 in cultured mouse astrocytes (Xiong Z *et al.*, 2014). A plausible explanation for the paradoxical results is that cognitive impairment in AD during the earlier stage may be attributed to decrease of IL-33 protein in association with downregulation of the IL-33 mRNA but that in the progressive stage of AD the IL-33 translation may be stimulated as compensative machinery. It remains to be explored whether IL-33 is good or bad for AD.

Fig 1. The expression levels of the IL-33 mRNA and protein in the hippocampus of 5xFAD mice



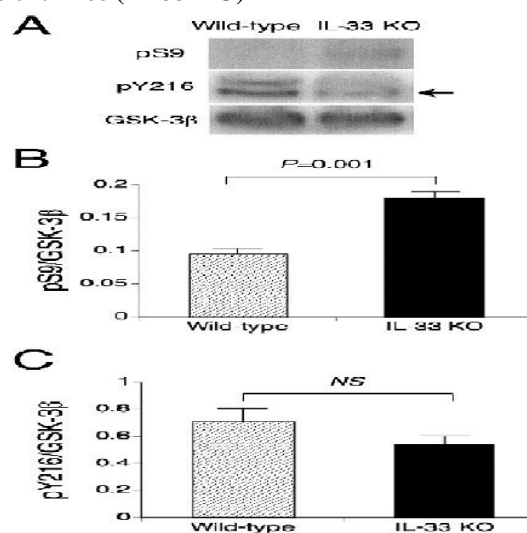
(A) Each column represents the mean (± SEM) IL-33 mRNA normalized by the GAPDH mRNA (n=4 independent mice). (B) Each column represents the mean (± SEM) IL-33 protein normalized by tissue protein weight (n=6 independent mice). *P* values, unpaired *t*-test.

Fig 2. Akt activity in the hippocampus of IL-33-deficient mice (IL-33 KO).

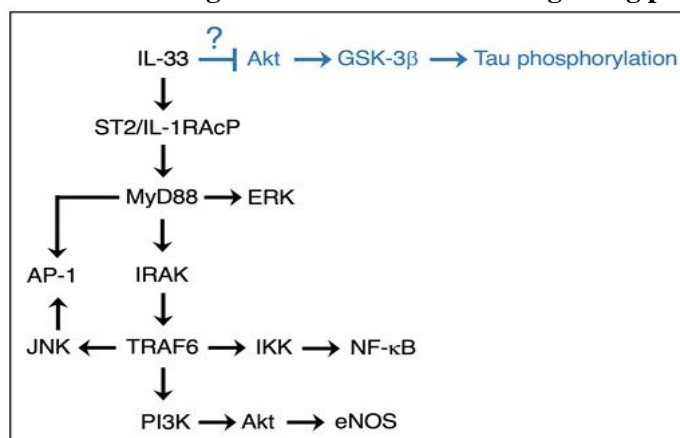


(A) Western blot image. (B) Each column represents the mean (± SEM) signal intensity for Thr308 phosphorylation relative to the intensity for Akt (n=4 independent mice). (C) Each column represents the mean (± SEM) signal intensity for Ser473 phosphorylation relative to the intensity for Akt (n=4 independent mice). *P* value, unpaired *t*-test. *NS*, not significant.

Fig 3. GSK-3β activity in the hippocampus of IL-33-deficient mice (IL-33 KO)



(A) Western blot image. An arrow indicates the signal band for GSK-3β phosphorylation at Tyr216. (B) Each column represents the mean (± SEM) signal intensity for Ser9 phosphorylation relative to the intensity for GSK-3β (n=4 independent mice). (C) Each column represents the mean (± SEM) signal intensity for Tyr216 phosphorylation relative to the intensity for GSK-3β (n=4 independent mice). *P* value, unpaired *t*-test. *NS*, not significant.

Fig 4. A schematic diagram for IL-33-mediated signaling pathways.**CONCLUSION**

The results of the present study demonstrate that the IL-33 protein levels rise in the hippocampus of 5xFAD mice, an animal model of AD, and that Akt activation and GSK-3 β inactivation are accelerated by deleting IL-33.

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Nil

CONFLICT OF INTEREST

No interest

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