

MICRO REPORT

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# The VEGF inhibitor vatalanib regulates AD pathology in 5xFAD mice

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## Abstract

Alzheimer's disease (AD) is a highly prevalent neurodegenerative disease characterized by A $\beta$  accumulation and tau hyperphosphorylation. Epidemiological evidence for a negative correlation between cancer and AD has led to the proposed use of tyrosine kinase inhibitors (TKIs) such as dasatinib and masitinib for AD, with reported beneficial effects in the AD brain. The TKI vatalanib inhibits angiogenesis by inhibiting vascular endothelial growth factor receptor (VEGFR). Although changes in VEGF and VEGFR have been documented in AD, the effect of vatalanib on AD pathology has not been investigated. In this study, the effects of vatalanib on tau phosphorylation and A $\beta$  accumulation in 5xFAD mice, a model of AD, were evaluated by immunohistochemistry. Vatalanib administration significantly reduced tau phosphorylation at AT8 and AT100 by increasing p-GSK-3 $\beta$  (Ser9) in 5xFAD mice. In addition, vatalanib reduced the number and area of A $\beta$  plaques in the cortex in 5xFAD mice. Our results suggest that vatalanib has potential as a regulator of AD pathology.

**Keywords:** Alzheimer's disease, Tau, Amyloid beta, 5xFAD mice, Vatalanib, Vascular endothelial growth factor, Tyrosine kinase inhibitor

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease. The accumulation of amyloid  $\beta$  (A $\beta$ ) plaques and neurofibrillary tangles (NFTs) in the brain is the neuropathological hallmark of AD. A $\beta$  plaques are formed from the accumulation of A $\beta$  produced by sequential cleavage of amyloid precursor protein (APP) via  $\beta$ - and  $\gamma$ -secretase, and NFTs are aggregates of hyperphosphorylated tau, which normally functions in microtubule stabilization. A $\beta$  plaque and NFT levels are both discriminators of and contributors to AD progression and are closely correlated with cognitive impairment in AD subjects [1]. Although AD and cancer share aging as a common factor, accumulating epidemiological evidence indicates a negative correlation between cancer and AD. This evidence has led to the proposed repurposing of cancer drugs of various mechanisms of

action for the treatment of AD [2]. These cancer drugs include tyrosine kinase inhibitors (TKIs) such as dasatinib, masitinib, and imatinib, which have been shown to have therapeutic effects on the pathogenesis of AD [3–5].

Vatalanib, a small-molecule anticancer drug that inhibits angiogenesis (Fig. 1a), is a broad-spectrum TKI that exerts its effects by occupying the ATP-binding sites of vascular endothelial growth factor receptor 1–3 (VEGFR1–3), platelet-derived growth factor receptor  $\alpha$  (PDGFR $\alpha$ ), and c-KIT [6]. VEGF and VEGFR have been implicated in angiogenesis as well as blood–brain-barrier (BBB) permeability and microglial chemotaxis in AD pathology [7–9]. VEGF levels are increased in the peripheral blood, cerebrospinal fluid, and microglia of patients with AD and are correlated with the clinical severity of AD [8, 10, 11], and VEGF and VEGFR expression are also increased in AD animal models [12]. However, the effect of vatalanib on tau phosphorylation and A $\beta$  accumulation in the AD brain has not been studied.

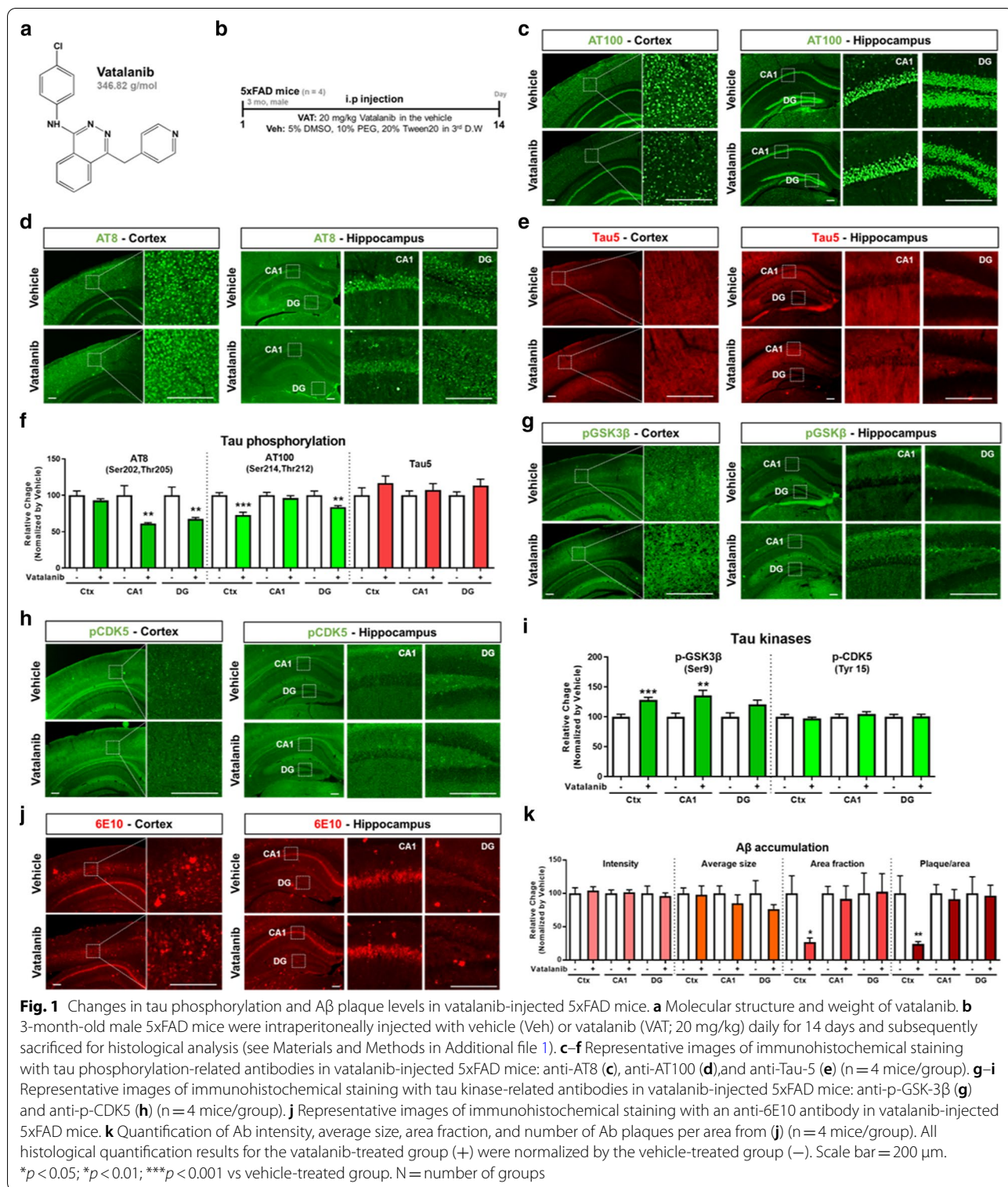
To investigate the effect of vatalanib on the AD brain, we administered 20 mg/kg of vatalanib daily for 14 days

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to 3-month-old 5xFAD mice, a model of AD (Fig. 1b). Eight hours after the last injection, the mice were fixed via sequential cardiac perfusion with PBS and 4% paraformaldehyde, and immunofluorescence staining was

performed using AT100 (Ser<sup>202</sup> and Thr<sup>205</sup>), AT8 (Ser<sup>214</sup> and Thr<sup>212</sup>), and Tau5 (total tau) antibodies to visualize tau phosphorylation (Fig. 1c–e). Histological quantification of the fluorescence intensity of tau phosphorylation

(Fig. 1f) revealed a significant decrease in AT8 immunoreactivity in the hippocampus (CA1 and DG) of vatalanib-treated 5xFAD mice compared with vehicle-treated 5xFAD mice (Fig. 1c, f). The fluorescence intensity of AT100 was also significantly decreased in the cortex and hippocampus DG of vatalanib-treated 5xFAD mice (Fig. 1d, f). However, the fluorescence intensity of Tau5 was not altered by the administration of vatalanib (Fig. 1e, f). Thus, vatalanib administration in 5xFAD mice significantly reduced phospho-tau<sup>Ser202, Thr205</sup> and phospho-tau<sup>Thr212, Ser214</sup> without altering total tau levels.

To investigate the molecular mechanism by which vatalanib modulates tau phosphorylation in 5xFAD mice, immunofluorescence staining was performed with antibodies against p-GSK-3 $\beta$  and p-CDK5, the major molecules involved in tau phosphorylation (Fig. 1g, h). Histological quantification of the fluorescence intensity of tau kinases (Fig. 1i) revealed a significant increase in p-GSK-3 $\beta$ <sup>Ser9</sup> in the cortex and hippocampus CA1 of vatalanib-administered 5xFAD mice (Fig. 1g, i). However, another major tau kinase, p-CDK5, was not altered by the administration of vatalanib in 5xFAD mice (Fig. 1h, i). It is well known that the constitutive activity of GSK-3 $\beta$  is inhibited by GSK-3 $\beta$  (Ser9) phosphorylation. Therefore, our results suggest that increased phosphorylation of GSK-3 $\beta$  (Ser9) may have contributed to the reduction of tau phosphorylation by vatalanib administration.

To investigate the accumulation of A $\beta$ , another histopathological feature of AD, immunofluorescence staining was performed in vatalanib-treated mice using a 6E10 antibody that labels A $\beta$ <sub>1–16</sub> as an epitope (Fig. 1j). To evaluate the effect of vatalanib on A $\beta$  accumulation in 5xFAD mice, the fluorescence intensity, area fraction and average size of A $\beta$  plaques and the number of A $\beta$  plaques per area were quantified based on 6E10 immunoreactivity (Fig. 1k). Although vatalanib administration did not alter the fluorescence intensity of A $\beta$  plaques in 5xFAD mice, the area fraction of A $\beta$  plaques in the cortex was significantly reduced. The number of A $\beta$  plaques per area in the cortex was also significantly reduced in vatalanib-administered 5xFAD mice. However, the average size of A $\beta$  plaques was not altered by vatalanib administration in 5xFAD mice (Fig. 1k).

In summary, we evaluated the effects of vatalanib on AD pathology in 5xFAD mice. Previous studies have demonstrated that both AT8- and AT100-labeled phospho-tau epitopes are elevated in the AD brain [13, 14]. In addition, 5xFAD mice have been reported to exhibit hyperphosphorylation of tau and decreased p-GSK-3 $\beta$  (Ser9) [15]. In the present study, administration of vatalanib attenuated tau phosphorylation at AT8 and AT100 in 5xFAD mice, and these changes were accompanied by increased GSK-3 $\beta$ <sup>Ser9</sup> phosphorylation (Fig. 1c–i). These

results indicate that vatalanib differentially regulates tau phosphorylation by modulating the GSK-3 $\beta$  phosphorylation epitope in 5xFAD mice. While vatalanib administration had no effect on A $\beta$  fluorescence intensity or A $\beta$  plaque size, the number of A $\beta$  plaques excluding intracellular A $\beta$  in the cortex was significantly decreased in 5xFAD mice (Fig. 1k). Considering that 3- to 4-month-old 5xFAD mice predominantly accumulate A $\beta$  in the deep cortical layer and subiculum and not the hippocampus, it is reasonable that vatalanib has an inhibitory effect on A $\beta$  accumulation in the cortex [16]. It is possible that long-term administration of vatalanib might inhibit A $\beta$  accumulation according to age. Interestingly, a recent study found that A $\beta$  oligomers activate microglial cells in a tyrosine kinase-dependent manner [17]. In addition, since VEGF and VEGFR are involved in BBB permeability and microglial chemotaxis, future studies will address whether vatalanib affects neuroinflammatory responses and BBB integrity in 5xFAD mice [7–9]. Moreover, we will evaluate the effects of vatalanib on tauopathy, basal behavior, and cognitive impairment in a mouse model of AD (e.g., PS19 Tau Tg or 5xFAD mice) and identify the mechanism by which vatalanib alters tau phosphorylation as well as synaptic function. In conclusion, intraperitoneal injection of vatalanib in 3-month-old 5xFAD mice reduced A $\beta$  plaque levels and tau phosphorylation, and thus vatalanib may be a drug candidate for Ab-and/or tau-associated diseases, including AD.

## Supplementary information

**Supplementary information** accompanies this paper at <https://doi.org/10.1186/s13041-020-00673-7>.

**Additional file 1.:** Materials and methods.

## Abbreviations

A $\beta$ : Amyloid  $\beta$ ; AD: Alzheimer's disease; APP: Amyloid precursor protein; CDK-5: Cyclin-dependent kinase-5; DG: Dentate gyrus; GSK-3 $\beta$ : Glycogen synthase kinase-3 $\beta$ ; NFT: Neurofibrillary tangle; PDGFR $\alpha$ : Platelet-derived growth factor receptor  $\alpha$ ; TKI: Tyrosine kinase inhibitor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor.

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## Authors' contributions

SGJ and HSH conceived and participated in the design of the study. SGJ, HJL, HP and KM performed *in vivo* experiments and histological analysis. SGJ and HSH wrote the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

All animal experiments were approved by the Institutional Animal Care and Use Committee at Korea Brain Research Institute (KBRI) (Assigned No. IACUC-2016-0013, IACUC-19-00049, IACUC-19-00042).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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