Monitoring HDAC4 Expression in Alzheimer's Disease Using [^{18}F]TFAHA-PET

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1 Introduction

Amyloid-β (Aβ) is widely considered as a key contributor to the pathophysiology of AD [1]. Despite various therapeutic approaches targeting Aβ through either secretase inhibitors or immunotherapy, the cognitive functions of patients failed to be

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improved effectively [2]. Recent findings indicated the abnormal epigenetic modifications contribute to the impaired learning and deterioration of memory [3]. Epigenetic dyregulation causes the widespread decline in gene expression through post-translational histone modifications including epigenetic modifying enzymes, histone deacetylases (HDACs) [4]. Among these histone-modifying enzymes, HDAC4, belongs to HDAC class IIa enzymes, is highly enriched in brain and its homeostasis is associated with regulating the transcription of synaptic plasticity-related gene, neuronal survival and neuron development [5]. Studies have shown that the nuclear HDAC4 level is markedly increased in brains of AD patients and the total HDAC4 levels are highly expressed in AD mouse model [6, 7]. It suggests that abnormal HDAC4 expression or its nuclear localization may contribute to learning and memory deficits.

Given that the importance of HDAC class IIa in epigenetic regulation involved in brain function and HDAC inhibitor treatments restore cognitive performance, it is necessary to develop molecular imaging agents for evaluation of HDACs activity in the brain. Consequently, this has led to the development of a second generation of HDAC class IIa–specific radiotracer, 6-(tri-fluoroacetamido)-1-hexanoicanilide ([^{18}F]TFAHA), which shows significantly higher selectivity for HDAC class IIa enzymes [8]. Recent study has described that PET imaging with [^{18}F]TFAHA enable quantitative assessment of HDAC class IIa enzymes expression-activity in intracerebral 9L and U87-MG gliomas in rats [9]. However, whether PET imaging with [^{18}F]TFAHA can be used to monitor HDAC class IIa enzymes in AD brain needs to be investigated.

The aim of this study is to investigate the role of HDAC4 using 3D human neural cell model of AD by analysis of expression and treatment of specific inhibitor. We also evaluate HDAC4 expression in 3xTg AD mice compared with age-matched WT mice by performing PET imaging with [^{18}F]TFAHA and immunohistochemical analysis. The results suggest that [^{18}F]TFAHA is a useful tool for in vivo monitoring epigenetic deregulation, especially HDAC4, in AD progression.

2 Materials and Methods

A. Establishment of 3D human neural cell culture model of AD and relevant assays

3D culture models were set up for subsequently IF and biochemical analysis according to the method described previously [10]. To further investigate the effects of Tasquinimod on neuronal memory and synaptic plasticity-related genes, 3D-differentiated FAD cells were treated with Tasquinimod at doses between 30 and 100 μM for 48 h. Subsequently, RT-qPCR for gene expression was performed.

B. Animals

3xTg-AD mice (JAX-34830) and age-matched control group were used in this study. All animal procedures were performed in accordance with the institutional guidelines for care and use of laboratory animals.

C. Small animal PET/CT imaging experimental procedures

In PET image study, each AD mouse and age-matched WT mouse was injected intravenously with 8.04 ± 0.75 MBq/0.1 ml of [^{18}F]TFAHA. Subsequently, regional retention and uptake of these radioligands were processed and analyzed with PMOD 3.5 software package (Pmod Technologies, Zu¨rich, Switzerland).

D. Immunofluorescent staining

Brain Sects. (10 μm) or cell culture slides were incubated with the primary antibodies followed by fluorescence conjugated secondary antibodies (Abcam) and DAPI. All slides were observed under a confocal fluorescence microscopy (Zeiss LSM 880).

E. Statistical analysis

Data were statistically analyzed with GraphPad Prism (Version 5.0; GraphPad, La Jolla, USA) using Student's *t* test or by a one-way ANOVA, followed by Tukey's post hoc test.

3 Results

A. In vivo monitoring of HDAC4 using [^{18}F]TFAHA in 3xTg AD

To monitor HDAC4 expression in AD progression, we performed PET imaging with [^{18}F]TFAHA in AD transgenic mouse and age-matched wild type mouse. 3xTg AD mouse model, which exhibits progressive Aβ deposition and age-related changes in neuropathologies, is suitable to observe the changes of HDAC4 with age. Visual interpretation of [^{18}F]TFAHA-PET results showed greater uptake of [^{18}F]TFAHA in the whole brains of 3xTg AD mice compared with age-matched WT mice at 8, 11 and 16 months of age (Fig. 1a–c). Higher uptake were found in 3xTg AD mice consistently, suggesting that [^{18}F]TFAHA-PET imaging could be used to differentiate AD and WT control.

All brains were further anatomically parcellated according to a standard atlas and [^{18}F]TFAHA uptake in each region of cortex, hippocampus, striatum, basal forebrain, thalamus and cerebellum was evaluated as for whole brain analysis (Fig. 2). The 3xTg AD mice exhibited significantly higher [^{18}F]TFAHA uptake in most areas examined, including hippocampus ($t = 3.45$, $p = 0.04$), basal forebrain ($t = 5.61$, $p = 0.0112$), thalamus ($t = 4.36$, $p = 0.0224$) and cerebellum ($t = 2.38$, $p = 0.146$) compared to wild-type mice. Despite the [^{18}F]TFAHA uptake in neither cortex ($t = 2.67$, $p = 0.12$) nor striatum ($t = 4.06$, $p = 0.056$) reached statistical significance, both of these two regions of 3xTg AD mice showed higher [^{18}F]TFAHA uptake.

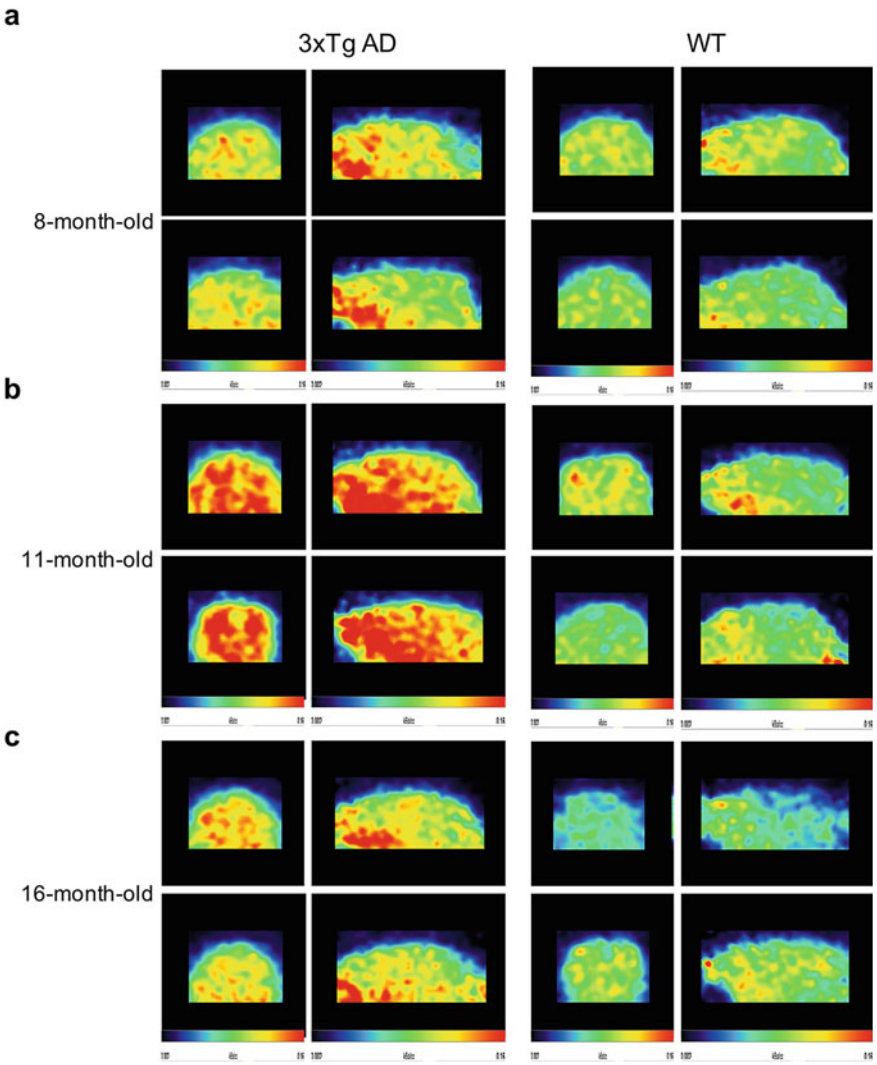

Fig. 1 In vivo PET imaging of HDAC4 using [^{18}F]TFAHA. Representative [^{18}F]TFAHA PET imaging of 3xTg AD mouse and WT mouse at **a** 8, **b** 11, **c** 16 months of age

To investigate whether the level of HDAC4 coincides with the uptake of [^{18}F]TFAHA, immunostaining with HDAC4 specific antibody was performed in brain sections. In line with the PET studies, differences in levels of HDAC4 were visually detectable between 3xTg AD and wild-type mice (Fig. 3).

B. In vitro characterization of HDAC4 expression in cell model of Alzheimer's disease

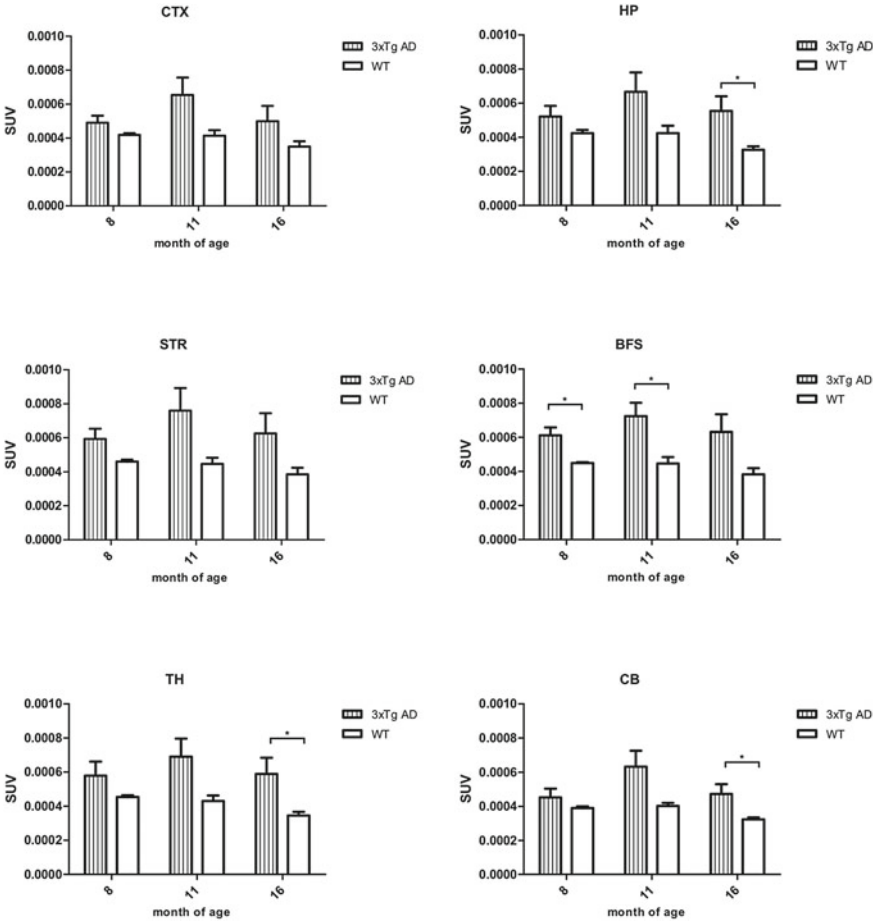

Fig. 2 The quantification of whole brain and different brain regions in SUV. SUV means standardized uptake value. Abbreviation: CTX, cortex; HP, hippocampus; STR, striatum; BFS, basal forebrain; TH, thalamus; CB, cerebellum

To investigate the HDAC4 expression following neuronal differentiation, a human neural cell culture model of AD that mimicked AD pathology by overexpressing human amyloid precursor protein with both Swedish (K670N/M671L) and London (V717I) FAD mutations in SH-SY5Y cell line was used (namely 3D-differentated FAD cells) in this experiment. IF staining and western blot analysis showed that HDAC4 was increased in 3D-differentated cells during neuronal differentiation either FAD or WT (Fig. 4a). By contrast, the level of HDAC1 remained largely unaffected. In addition, increased Hdac4 at the mRNA level was found in 3D-differentated FAD cells (Fig. 4b).

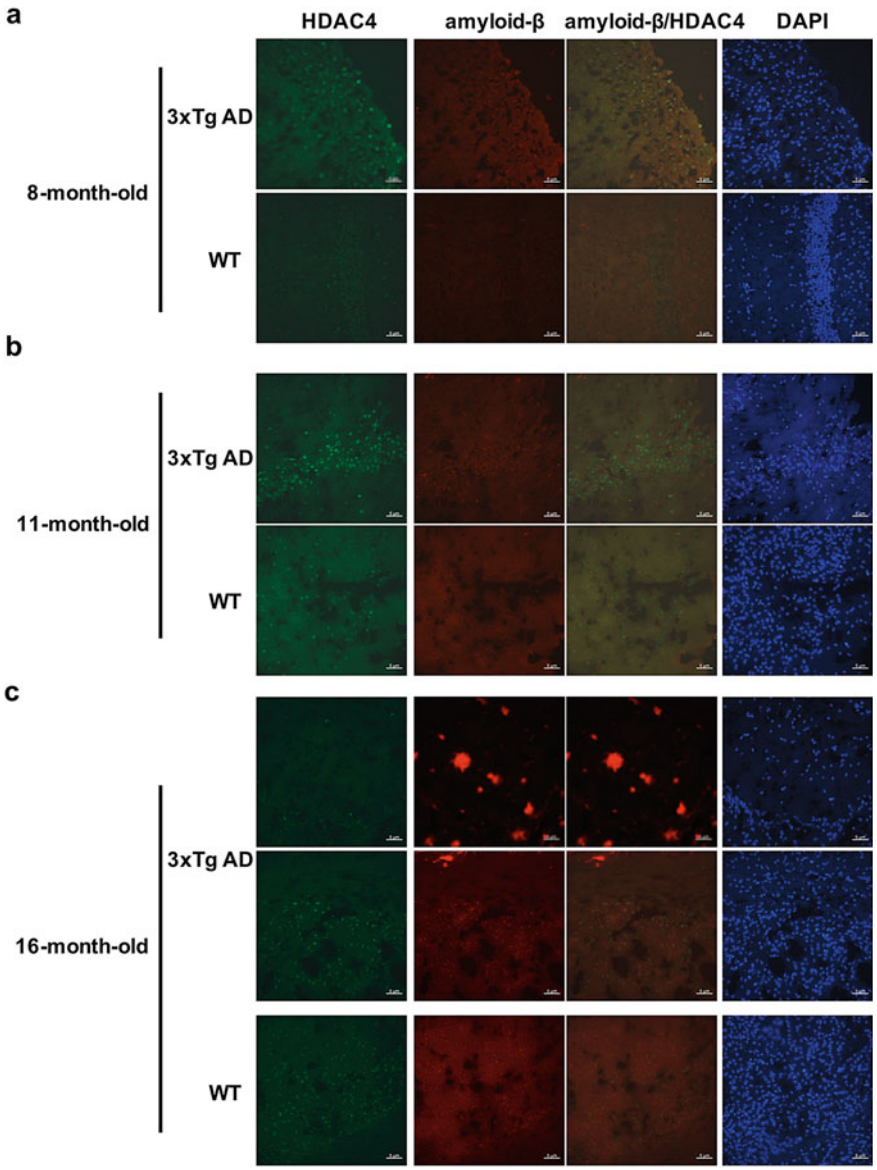

Fig. 3 Expression of HDAC4 and amyloid-β were increased in cortex of 3xTg AD mouse

C. HDAC4 inhibitor treatment up-regulates memory and synaptic plasticity-related genes

Given that the level of HDAC4 was elevated in cell model of AD, whether inhibition of HDAC4 can restore the levels of AD related affected genes is worthy of pursuit.

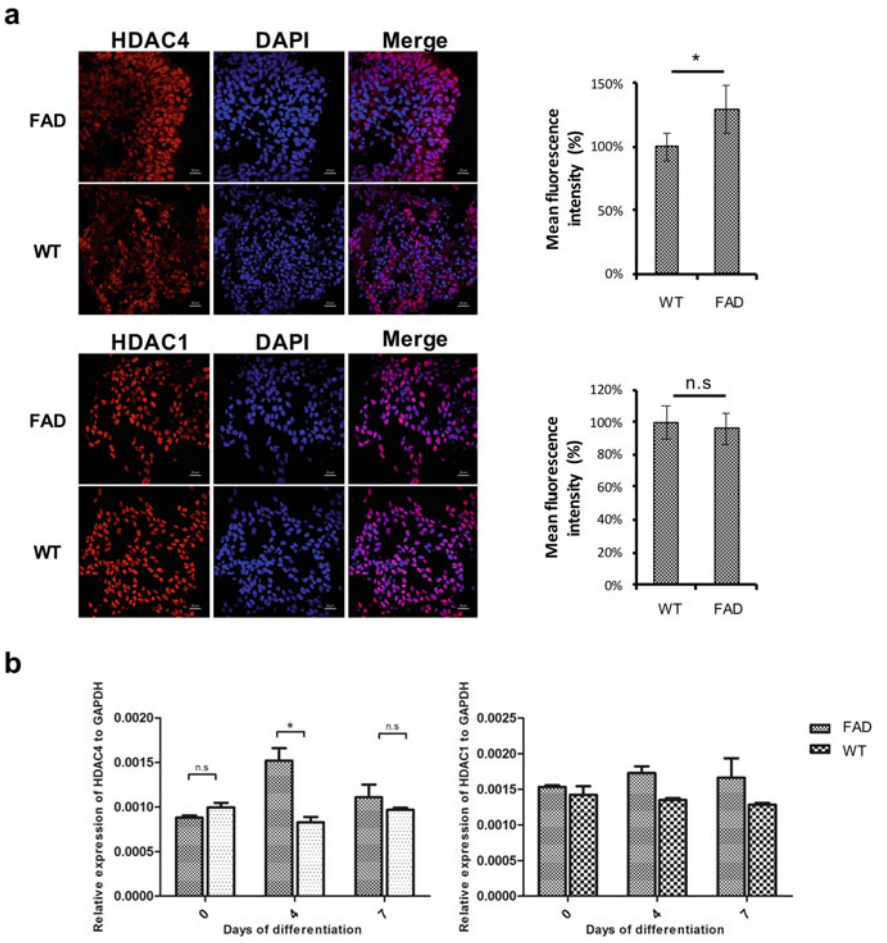

Fig. 4 The levels of HDAC4 are increased in 3D neural cell culture of AD

Tasquinimod (TasQ) is a highly specific inhibitor of HDAC4 and clinically tested oral antiangiogenic agent for therapy of castration-resistant prostate cancer. Herein, our study aimed to investigate the effect of TasQ on neuronal cells in AD. Quantitative RT-PCR analysis showed that TasQ upregulated several AD related affected genes and even dose-dependently increased the levels of Syn2, Homer1 and GluR2 (Fig. 5). Among these AD related affected genes, Lgi1, Syn2 and Homer1 belong to HDAC4 target genes [5]. Taken together, these data demonstrated that HDAC4 inhibitor treatment exhibited considerable therapeutic benefits on neuronal memory and synaptic plasticity-related genes in AD.

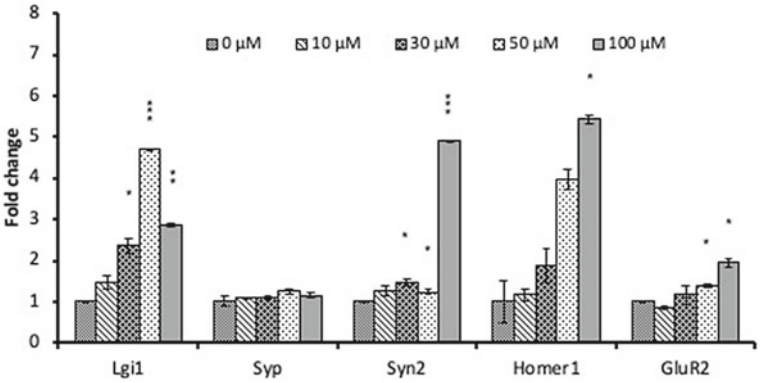

Fig. 5 HDAC4 specific inhibitor up-regulates memory and synaptic plasticity-related genes in SH-SY5Y FAD cells

4 Discussion

In this study, we demonstrated the important role that HDAC4 plays in Alzheimer's disease by analysis of its expression and treatment with specific inhibitor. These results validated that HDAC4 might be a promising for diagnosis and treatment of AD.

Despite higher uptake were found in 3xTg AD mice consistently, [^{18}F]TFAHA uptake was not positively correlated with the age (Fig. 4). We assumed that decreased level of HDAC4 at 16 months of age was attributed to neuronal loss. However, 3xTg AD mice were confirmed to expresses Aβ plaques and neurofibrillary tangles, as well as synaptic and behavioral deficits, except display significant neuronal loss [11]. [^{18}F]TFAHA-PET plus NeuN immunoreactivity and [^{18}F]FDG-PET to characterize AD pathology in 3xTg AD mouse model, may facilitate the development of AD research.

The variability of brain [^{18}F]TFAHA uptake at different age groups of AD mice was confirmed in current study. However, the progressive change of HDAC4 expression occurs in the same subject remains unknown. Thus, it is worthwhile to conduct longitudinal studies in mouse models of AD to better monitor and understand the events of epigenetic dysregulation throughout the course of the disease. Our data have shown that the level of HDAC4 coincides with the uptake of [^{18}F]TFAHA globally. However, owing to the limitation of μPET imaging for mouse brain, autoradiography assays can be used to evaluate the regional distribution and density of [^{18}F]TFAHA binding sites in the brains of the same mice that were imaged using PET.

There is growing evidence that aberrant HDAC expression and function leads to neuropathology. Previous studies showed that HDAC inhibitor specifically targeting class I, but not class IIa or IIb HDACs can significantly improve learning, memory and synaptic plasticity. Surprisingly, our results provided the first evidence that HDAC4 specific inhibition is also beneficial to AD related gene deficits. The therapeutic

effect of HDAC4 inhibition in AD transgenic mice needs to be investigated in terms of behavior and neuronal function.

5 Conclusions

Current studies demonstrated that brain [^{18}F]TFAHA uptake at different age groups of AD mice coincided with the level of HDAC4. Moreover, a significant therapeutic effect of HDAC4 specific inhibitor on neuronal cells was demonstrated by upregulation of memory and synaptic plasticity-related genes. Furthermore, PET imaging with novel histone deacetylase class IIa selective substrate-based radiotracer, [^{18}F]TFAHA revealed HDAC4 expression in aging AD brains, which may encourage the development of AD-related neuroimaging and therapy.

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Conflict of Interest The authors declare that they have no conflict of interest.

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