이학석사 학위논문

Characteristic glial responses to tau- or Aβ-containing lysates injection in tau transgenic mice

타우 형질전환쥐에서 타우단백질 또는 베타아밀로이드 함유 용해물 주사에 따른 특이적인 신경아교세포 반응

2021 년 8 월

서울대학교 융합과학기술대학원
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권 석 준

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2021 년 7 월

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권 석 준

권석준의 이학석사 학위논문을 인준함
2021 년 7 월

위 원 장 _____ 신 영 기

부위원장 _____ 이 동 수

위 원 _____ 최 유 리

Abstract

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In Alzheimer's disease (AD), pathogenic tau and Aβ have been presumed as culprit for AD pathogenesis. The accumulation of hyperphosphorylated tau into neuron or cell-to-cell propagation is common pathological features of AD, and these changes are closely related to cognitive impairment. However, since pathological changes in AD originate from Aβ, I try to identify the effects of Aβ on tau pathology by identifying the characteristics of the Aβ-induced tau model compared to the tau-induced tau model. Here, to understand distinct pattern of glial cells on tau pathology, brain lysates containing pathogenic tau or Aβ were intracerebrally inoculated into tau transgenic mice. Tau- or Aβ-induced tau models were used to validate induction of tau pathology and investigate differences in reactive glial cells. In the two tau propagation models, the rapid propagation and hyperactive behavior were observed at 1, 2, and 3 months after seeding, but the activation patterns of glial cells were different. Seeded tau induced microglial activation, whereas seeded Aβ immediately induced astrocytes activation and then decreased. These results showed that Aβ could induce changes in tau pathology and that there may be a difference in the mechanisms by which Aβ and tau induce tau pathology. Therefore, these models are able to characterize tau pathology in AD and have

shown that initiation and propagation might be induced differently in AD compared to tauopathy.

Key words : Alzheimer's disease, tau protein, β-amyloid, glial activation

Student Number : 2019-23996

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Introduction

The microtubule-associated protein tau (MAPT) is widely known to physiologically regulate stability of microtubules by binding to them. As exons 2, 3 and 10 of tau gene are alternatively spliced, there are six major tau isoforms. Tau has no N-terminal inserts, exon 2 or exon 2 and 3. They also differ in their microtubule-binding repeat domains (tau with 3 repeats or 4 repeats) (Tapia-Rojas et al., 2019; Chong et al., 2018). A number of posttranslational modification (PTM) of tau have been observed including phosphorylation, acetylation, glycosylation, nitration and methylation, etc. Among such modifications, phosphorylation is the most studied PTM of tau (Bramblett et al., 1993; Mandelkow et al., 1995; Mi and Johnson, 2006). Several studies have established that tau affinity for microtubules is reduced by tau hyperphosphorylation (Buee et al., 2000; Hanger et al., 2009; Sergeant et al., 2008). However, there are a large number of sites for phosphorylation, implicating differential effects on tau function. Therefore, various isoforms and posttranslational modifications of tau suggest involvement in distinct functions. In addition to stabilizing microtubule, the tau is currently presumed to participate in diverse biological processes including axonal transport, synaptic activity, nuclear activity. However the functions of tau in physiological conditions remain to be defined (Chang et al., 2021).

The tau has also been implicated in neurodegenerative disorders such as tauopathy. Tauopathies that include Pick's disease (PiD), Progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD), chronic traumatic encephalopathy (CTE) and Alzheimer's

disease (AD) are characterized by the accumulation or propagation of pathological tau. Although the pathological forms of tau have not yet been clearly identified (Kaufman et al., 2016; Goedert and Spillantini, 2019), in such diseases, some PTMs have been presumed to be a crucial factor which induces tau aggregation. Especially in tau hyperphosphorylation, hyperphosphorylated tau protein is given conformational changes that are conversion of monomer to oligomer and aggregation into paired helical filaments (PHFs) formable to neurofibrillary tangles (NFTs). Abnormal tau aggregation and propagation are a causative factor in tau burdening diseases. Impaired locomotion is found in tau transgenic mice, and in AD, incremental phosphorylated tau rather than Aβ is correlated with cognitive deficits. In addition, excessively and abnormally phosphorylated tau lead to destabilized microtubule network, synaptic deficits and then neural death. Therefore, It is necessary to scrutinize abnormal tau phosphorylation and aggregation to develop therapeutic strategy for tau related neurodegenerative diseases.

Although tau is an intracellular protein, they also present in extracellular space. The extracellular tau has been considered to be culprit of tau seeding and propagation along connected neurons, though its physiological functions remain enigmatic. The exact mechanisms of tau secretion and their prion-like propagation remain unclear (Brunello et al., 2020; Prusiner, 2012; Goedert et al., 2017). To investigate the importance of tau seeding and propagation, numerous researchers have used tau transgenic mice overexpressing human tau isoforms or have performed inoculation of several types of tau as “seeds” such as synthetically prepared forms and brain tissue-derived forms into mouse brain since 2009 (McAllister et al., 2020; Clavaguera et al., 2009). These models exhibit age-associated

progression of frontotemporal lobal degeneration (FTLD). Commonly used tau transgenic mice such as P301L or P301S exhibit their slow progression with behavior abnormality and tau pathology. Thus, It is efficient to use tau inoculation model with rapid tau propagation. The tau inoculation curtail the onset of tau pathology (McAllister et al., 2020). The intracerebral seeding experiments, that is, have provided crucial clues of pathogenesis from fixed time point and designated region of seeding. It has been demonstrated that seeded tau not only internalize within neurons or other glial cells but also induce propagation along functionally connected neurons concurrent with progressive and stereotypical accumulation of hyperphosphorylated tau (Ahmed et al., 2014; Gibbons et al., 2017; Stancu et al., 2015; Boluda et al., 2015). Therefore, the tau seeding model for investigation of tau pathology would be more practical approach rather than intact transgenic model.

These tau pathology is closely related to cognitive function. During progression of mild cognitive impairment to dementia, tau-mediated neural injury is correlated with cognitive impairment (Masters et al., 2015). In Alzheimer's disease, Aβ and tau are presumed as culprit, since in the brains of patients with AD, extracellular amyloid plaques and intracellular NFTs are histopathological characteristics. However, the onset of cognitive decline depends on co-existence of tau and Aβ, not tau alone (Betthauser et al., 2020). In healthy neurons, amyloid precursor protein (APP) is digested by α-secretase and γ-secretase enzymes, producing soluble polypeptides. But, when the β-secretase teams up with the γ-secretase, an insoluble peptide called amyloid-β ($A\beta_{1-42}$) is produced which can clump together and form amyloid plaques (Ashrafian et al., 2021). The amyloid-cascade hypothesis has provided the understanding for pathogenesis of both familial and sporadic AD, since its introduction in

1991 (Hardy and Allsop, 1991; Selkoe, 1991). This hypothesis suggest neuropathological changes in Aβ as cause of AD. The hypothesis has evolved for many years. It provides an alternative model proposes contribution of Aβ and tau pathology through correlated (not Aβ alone) but independent cellular pathways to neurodegeneration of AD (Selkoe and Hardy, 2016). For many years, accumulating evidences, however, support that pathologies of tau and Aβ have synergistic effects. Aβ-dependent neuronal hyperexcitability was not only blocked but also suppressed by the presence of tau (Busche et al., 2019). And amyloid plaques facilitated neuritic plaque tau aggregation (He et al., 2018). But, understanding of the interaction between tau and Aβ needs to be more investigated.

In many neurodegenerative disease, neuroinflammation characterized by activation of glial cells has been presumed as cause of disease. Glial cells such as microglia, astrocytes are known to protect the brain parenchyme from pathogens. Microglia is a brain-resident immune cells. Especially microglia play critical roles in neurodegenerative and neuroinflammatory diseases of CNS as well as in neural development and homeostasis. Recently advanced transcriptome analyses revealed specific region- and time-dependent subtypes of microglia that can play specific functions (Masuda et al., 2019; Li et al., 2019). Understanding spatiotemporal heterogeneity of microglia provides development of investigation for underlying mechanisms or innovative therapies. Microglia known as innate immune cells are now accepted that they have innate immune memory, that is, they can enhance or suppress their responses to a secondary, delayed insult (Neher, 2019). When microglia are exposed to initial stimulus, microglial response is altered to a stronger reaction (immune training) or weaker reaction (immune tolerance) according to subsequent inflammatory stimuli. The

precise microglial response and contribution to tau pathology are unclearly investigated. Several studies demonstrated that microglial fractalkine receptor (CX3CR1) negative reactive microglia drive tau pathology and memory impairment in mouse model (Maphis et al., 2015; Bolos et al., 2017). Activated NLRP3 inflammasome in microglia that consists of NLRP3, caspase-1 and ASC leads to tau pathology (Ising et al., 2019). To investigate microglial contribution to tau pathology, several studies have used colony-stimulating factor receptor 1 (CSF1R) inhibitor to reduce or delete the number of microglia in mouse brain. The depletion of microglia by JNJ-527 (CSFR1 inhibitor) prevented tau pathology and locomotor behavior abnormality (Mancuso et al., 2019; Asai et al., 2015). However, partial reduction of microglia by 30 % did not attenuate tau burden (Bennett et al., 2018). Therefore, sufficient study for microglial involvement on progression of tau pathology remains to be defined.

In addition to microglia, astrocytes are thought to play a number of active roles, including the secretion or absorption of neural transmitters and maintenance of the blood-brain barrier. They share cytoplasm through gap junction as a syncytium, building a reticular network (Kiyoshi and Zhou, 2019). Astrocytes vary substantially in spatial organization along cortical neuron layers. Bayraktar et al. (2020) identified distinct gradient layer patterns of superficial and deep astrocytes with neuronal layers in cerebral cortex by using a single-cell in situ transcriptomic map (Bayraktar et al., 2020). Reminiscent of microglia, the precise astroglial reaction and contribution to tau pathology are unclearly investigated, although it is known that they become reactive under inflammation, acute injury and neurodegeneration.

Here, to understand differences between tau pathology induced by seeded tau and Aβ, brain lysates containing pathologic tau or Aβ from P301L or 5XFAD respectively were seeded into P301L human tau-expressing mouse. I validate the rapid tau propagation models with tau hyperphosphorylation and abnormally hyperactive behavior in tau or Aβ seeded groups. Interestingly, the activation pattern of glial cells was different between two tau propagation models. Seeded tau induced the activation of microglia, whereas seeded Aβ immediately induced astrocytes activation at 1 months after seeding and then decreased to 3 months after seeding. These characterization of two models may be crucial not only to elucidate disease pathogenesis in AD, but also to inform the necessity of astrocytic activation as diagnostic biomarker for progression of AD.

Materials and Methods

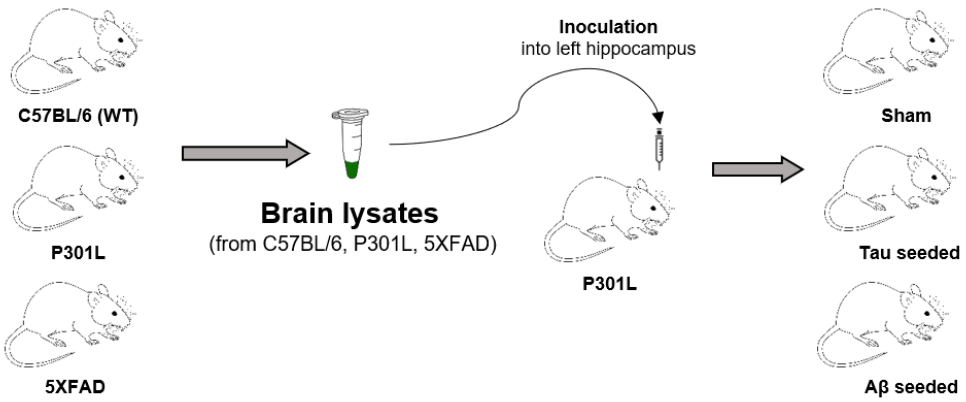
Animals

hTau.P301L homozygous (P301L) mice were maintained on a mixed C57BL/6, DBA/2 and SW background, expressing a 2N4R with a P301L mutation. 5XFAD mice were also bred on a mixed C57BL/6, SJL background, expressing human APP and PSEN1 transgenes with a total five AD-linked mutations and age matched littermates were used as wildtype (WT) mice. 21 and 15 P301L were randomized per lysates injected-group at 8.5 and 3 months respectively. For extract of brain lysates, 15 months old P301L, 5XFAD and WT mice were also maintained. In this study, all experiments were conducted with approval of Institutional Animal Care and Use Committee at Seoul National University.

Experimental design and grouping

To produce an accelerated and progressive tau pathology model, Brain lysates as the seeds were prepared from brains of C57BL/6, P301L and 5XFAD (n=5 per group), as described in part of materials and methods (Fig. 1a). Then, each lysate was unilaterally infused into hippocampus of left hemisphere of P301L brain (sham, tau- and Aβ-seeded P301L; n=3, 9, 9 at 8.5 months and 4, 6, 5 at 3 months respectively). I examined behavior and pathology to observe aberrant behavior and progression of tau pathology respectively as represented by diagrammatic workflow (Fig. 1b).

a.


C57BL/6 (WT)
P301L
5XFAD
Brain lysates
(from C57BL/6, P301L, 5XFAD)
Inoculation
into left hippocampus
P301L
Sham
Tau seeded
Aβ seeded

b.

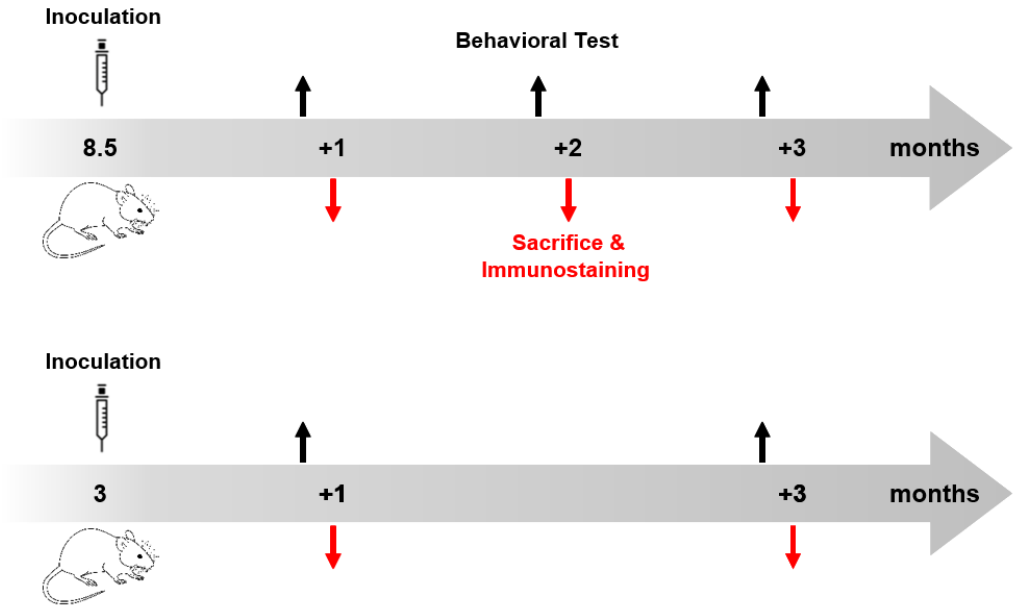

Inoculation
Behavioral Test
8.5
+1
+2
+3
months
Sacrifice &
Immunostaining
Inoculation
3
+1
+3
months

Figure 1. Graphical design of experiment

Diagrammatic representation of experiment. **a.** Brain lysates were prepared from brains of C57BL/6, P301L and 5XFAD (n=5 per group). Then, each lysates were unilaterally infused into left hippocampus of P301L brain. **b.** After intracerebral injection, behavior test and sacrifice for immunohistochemistry were performed monthly up to 3 months post-injection. 3 months aged younger mice were investigated at 1 and 3 months post injection.

Preparation of brain lysates

Brain lysates were prepared from P301L mice, age-matched 5XFAD and WT mice. In brief, mice were anesthetized with 2% isoflurane delivered in O_2 and perfused transcardially with cold Phosphate-buffer saline (PBS). Brains were extracted and rapidly frozen at -80°C. Brain stems were dissected from P301L mouse brains and cerebral cortices were obtained from 5XFAD and WT mouse brain. Brain extracts were homogenized at 10% w/v in PBS supplemented with a cocktail of protease and phosphatase inhibitors, followed by sonication (Branson 450, 0.9 seconds, 5 times) and centrifugation at 3,000 g for 5 minutes. The clear supernatants were aliquoted and stored -80°C still use.

Stereotaxic surgery

Mice were anesthetized with 2% isoflurane delivered in O_2 before immobilized in a stereotaxic frame. The skin was incised to expose the skull. They were received a unilateral injection of 2.5 µl each brain lysates into left hippocampus (Bregma: AP -1.2 mm; LM -1.2 mm; DV -2.45 mm; injected with 1.25 µl/minutes).

Immunoblotting

Brain lysates from all types of mice were boiled in 1X NuPAGE sample buffer [Invitrogen] for 5 minutes at 95°C. 100 µg/lane were loaded in 12% Tris-Glycine polyacrylamide gel, followed by transfer of proteins onto nitrocellulose membrane. After blocked in 5% milk diluted in Tris-buffered saline (TBS), membranes were immunoblotted with specific primary

antibodies (AT8 [Invitrogen; 1:1000], 6E10 [BioLegend; 1:1000], β-actin [Santa Cruz; 1:5000]). Visualization of proteins was achieved by using HRP-tagged secondary antibodies [Cell Signaling; 1:1000]. Optical densities were measured with Chemi image system [GE; Amersham 680]. And then, densitometry analysis was performed in ImageJBand. AT8 or 6E10 : β-actin ratio was normalized to densitometry values of β-actin.

Behavioral test

To monitor hyperactive, exploratory behavior and motor function deficits, I tested the mice (n=3 per group) in the open field. A video tracking system [EthoVision; Noldus] was used to track and assess several parameters; time spent in the center zone, total distance and distance traveled in the open/closed zone (total distance is only described in results). For test in open field, mice of each group were placed in a 80 cm X 80 cm square box. After allowed to explore the open zone freely for 30 minutes, total distance of them was assessed.

Immunostaining for phosphorylated tau and glial cells

All mice were perfused transcardially with cold sterile PBS and brains were extracted, postfixed overnight by immersion in 4% paraformaldehyde and then embedded in paraffin or OCT. In case of paraffin tissues, 4-μm-thick coronal sections (from Bregma 0 to -2 mm) were cut through the hippocampus using rotary microtomes. And frozen tissues were cut through 35-μm-thick sections. Then, sectioned paraffin brain samples were deparaffinized and rehydrated by immersion in xylene and series of graded ethanol respectively. Sections were

rinsed three times in distilled water (DW) and TBS followed by antigen retrieval using 0.01M of citric acid and then permeabilization with 0.5% TritonX-100 in 2.5X TBS for increase of antibody permeability. All paraffin and frozen sections were blocked in 5% Bovine serum albumin (BSA) in TBS for 60 minutes at room temperature followed by incubation in primary antibodies diluted in TBS. For detection of phosphorylated tau, sections were immunostained with monoclonal primary antibodies (pT231 [Abcam; 1:100] and AT8 [Invitrogen; 1:100]). Other primary antibodies for detection of glial cells are as follows: Iba1 [Abcam; 1:100], GFAP [Cell Signaling; 1:00], etc. Sections were rinsed three times in TBS followed by incubation in secondary antibodies diluted in TBS for 60 minutes at room temperature. Secondary antibodies used are as follows: Alexa Fluor 488 Goat anti-mouse IgG [Invitrogen; 1:200], Alexa Fluor 555 Donkey anti-rabbit IgG [Invitrogen; 1:200]. Sections were rinsed three times in TBS and mounted on microscope slides in mounting solution and coverslipped. Fluorescence images were acquired using the LEICA confocal microscopy SP8 and for the quantification of the fluorescence signal, MetaMorph Image Analysis Software [Molecular Devices] was used.

Statistical analysis

Prism GraphPad was used for visualization of data and data were summarized and presented as mean ± standard deviation (SD). An unpaired t-test was used to statistically analyze the groups in fluorometric assay and behavior test. Significant difference was assigned as $p < 0.05$ (*), $p < 0.005$ (**).

Results

Brain lysates from P301L and 5XFAD contain higher levels of phosphorylated tau and Aβ respectively

To investigate whether lysates of P301L and 5XFAD contain hyperphosphorylated tau and human Aβ respectively, samples were run on a western blot. After that, densitometry analysis was performed in Image J to quantify each band. AT8 recognizing phosphorylated paired helical filament tau was used to detect hyper-phosphorylated form. I found significantly higher level of AT8 positive signal in lysates of P301L than WT (Fig. 2a).

Aβ is composed of 36-43 amino acids whose sequences are contained with many fragments of Amyloid precursor protein (APP). I detected higher level of CTFβ (12 kDa), cleavage from APP, that is supposed to be degraded to Aβ by γ-secretase in 5XFAD overexpressing APP/PS1 than WT (Fig. 2b). Thus, lysates from P301L and 5XFAD have the potential to be used to induce tau pathology as seeds and lysate from C57BL/6 is sufficient for control of them.

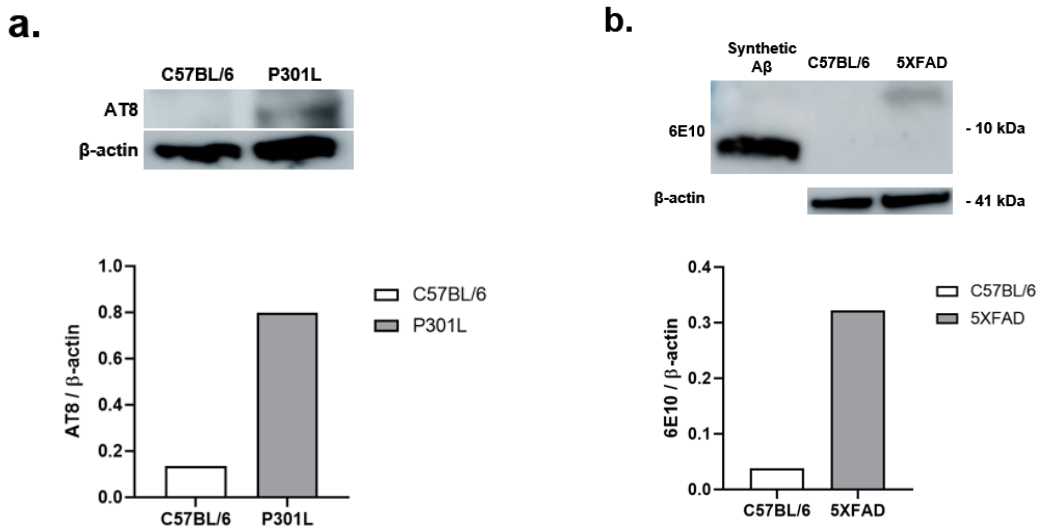


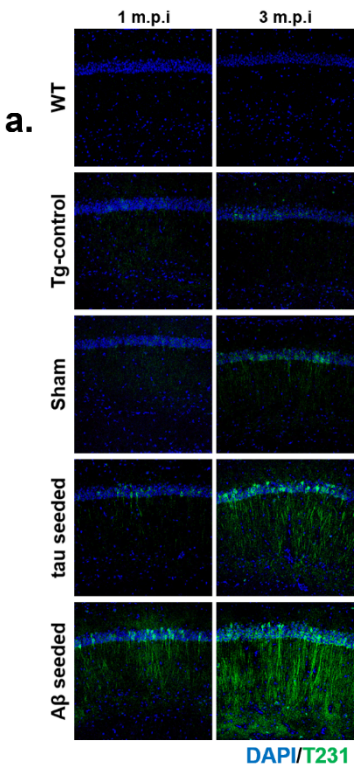
Figure 2. Brain lysates containing phosphorylated tau or human Aβ from P301L or 5XFAD respectively

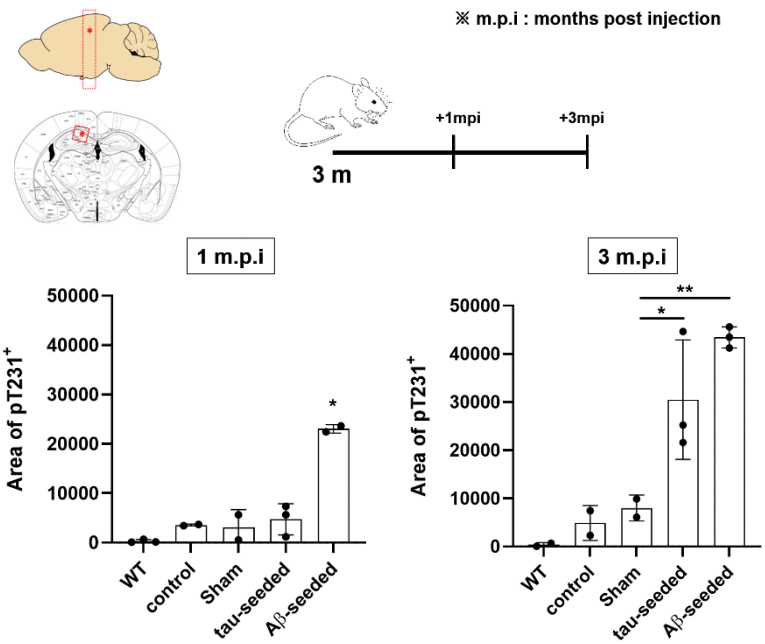
a, b. Representative images of western blot and quantification of densitometry. **a.** Brain lysates from P301L contained more AT8 positive phosphorylated tau than C57BL/6 normal mice. **b.** Brain lysates from 5XFAD contained more 6E10 positive APP fragments (CTFβ) than C57BL/6.

Seeded tau or Aβ induced rapid tau propagation

It has demonstrated that tau hyperphosphorylation and propagation are enhanced by inoculation with pathologic tau or even Aβ. To examine tau pathology, immunostaining was performed to monitor hyperphosphorylation of tau in hippocampus using antibody pT231 at time points of 1, 2 and 3 months post-injection (Fig. 3). I found the significantly elevated and gradually increasing level of pT231 positive tau in hippocampus of tau and Aβ seeded group compared to WT and sham group up to 3 m.p.i (Fig. 3a, b left panel). Interestingly, seeded tau in aged mice facilitated more rapid tau progression compared to younger mice.

To validate tau propagation, posterior side from injected site was investigated (Fig. 3b right panel). Elevated hyperphosphorylation of tau was found in posterior hippocampus at 3 m.p.i, suggesting that phosphorylated tau has been propagated along neural circuit. Therefore, I demonstrated that the rapid tau propagation models by injection of tau or Aβ were produced and validated.





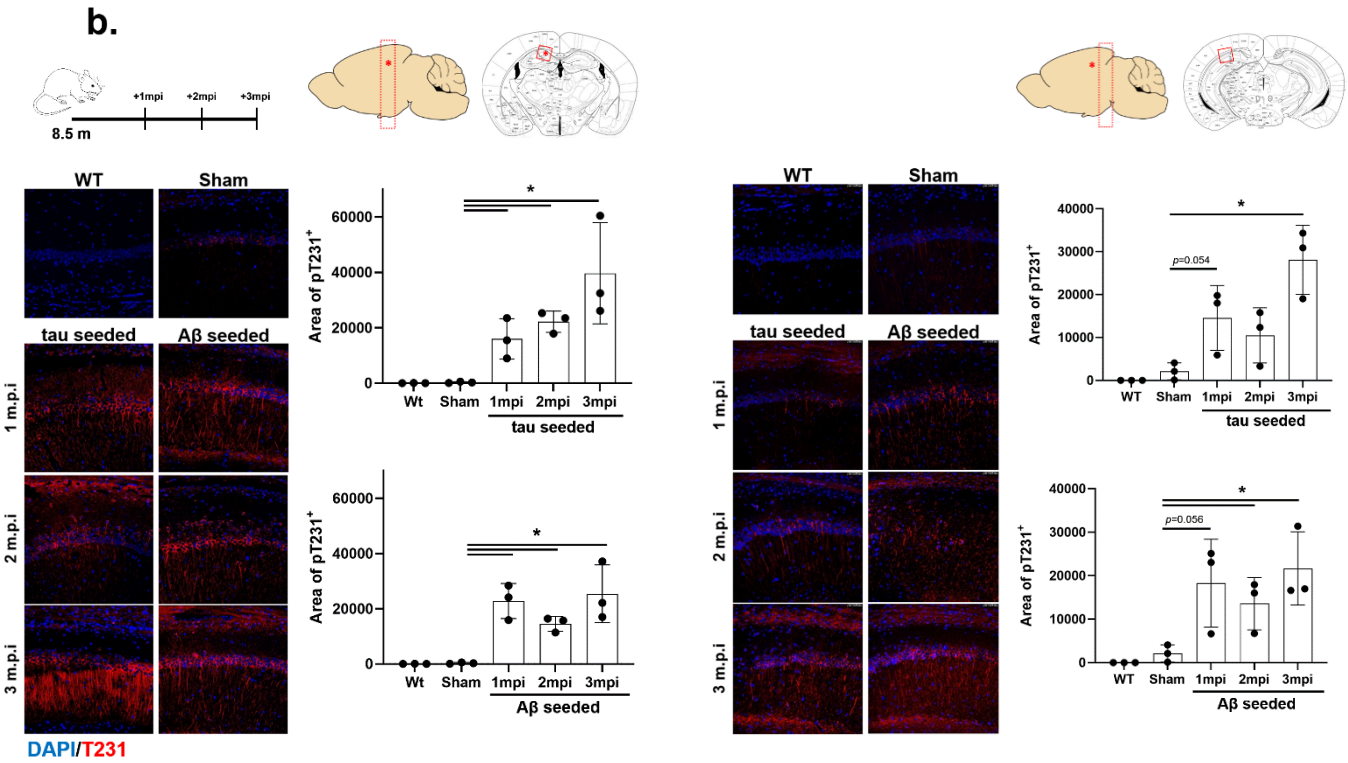

b.
+1mpi
+2mpi
+3mpi
8.5 m
WT
Sham
tau seeded
Aβ seeded
1 m.p.i
2 m.p.i
3 m.p.i
DAPI/T231
Area of pT231+
Wt
Sham
1mpi
2mpi
3mpi
tau seeded
Aβ seeded
p=0.054
p=0.056

Figure 3. Validation of rapid tau propagation model with tau hyperphosphorylation

a. Analysis of younger mice injected at 3 months of age. Histological analysis of coronal section of injected side (Bregma: AP -1.2 mm). Representative merged images of pT231 positive phosphorylated tau (pT231, green) and nucleus (DAPI, blue) immunostaining in hippocampus of WT, Tg-control, sham, tau- and Aβ-seeded group. Quantification of the area of pT231 positive tau of a left panel. **b.** Analysis of older mice injected at 8.5 months of age. Histological analysis of coronal section of injected and posterior side (Bregma: AP -1.2, -2 mm). Quantification of the area of pT231 positive tau. Data were expressed as AVE±SD ($P < 0.05$ (*), $P<0.005$ (**)). The m.p.i stands for months post injection.

P301L mice infused with lysates containing tau and Aβ aggregates presented increased hyperactive behavior in open field

Human mutant tau transgenic mice such as P301L and P301S present with a hyperactive phenotype correlated with progression of tau pathology (Jul et al., 2016). And It is known that cooperation with Aβ and tau causes a hyperactivity behavioral phenotype (Pickett et al., 2019). Thus, I further tested whether their behavior can be changed in open field after inoculation of tau and Aβ (Fig. 4). Total distances in open field were normalized to WT (Fig. 4a). both tau and Aβ seeded group show incremental hyperactive behavior compared to WT and sham group (Fig. 4a). Regression line of values of total distance was described along months post-injection (Fig. 4b). Considering decremental activity in WT along months post-injection, behavioral changes of both tau and Aβ seeded group are sufficient to represent the impaired behavior. Higher distance of sham group than WT seems to be due to difference between transgenic and non-transgenic strain. The result of behavior test support that seeded tau and Aβ induce hyperactive phenotype of P301L.

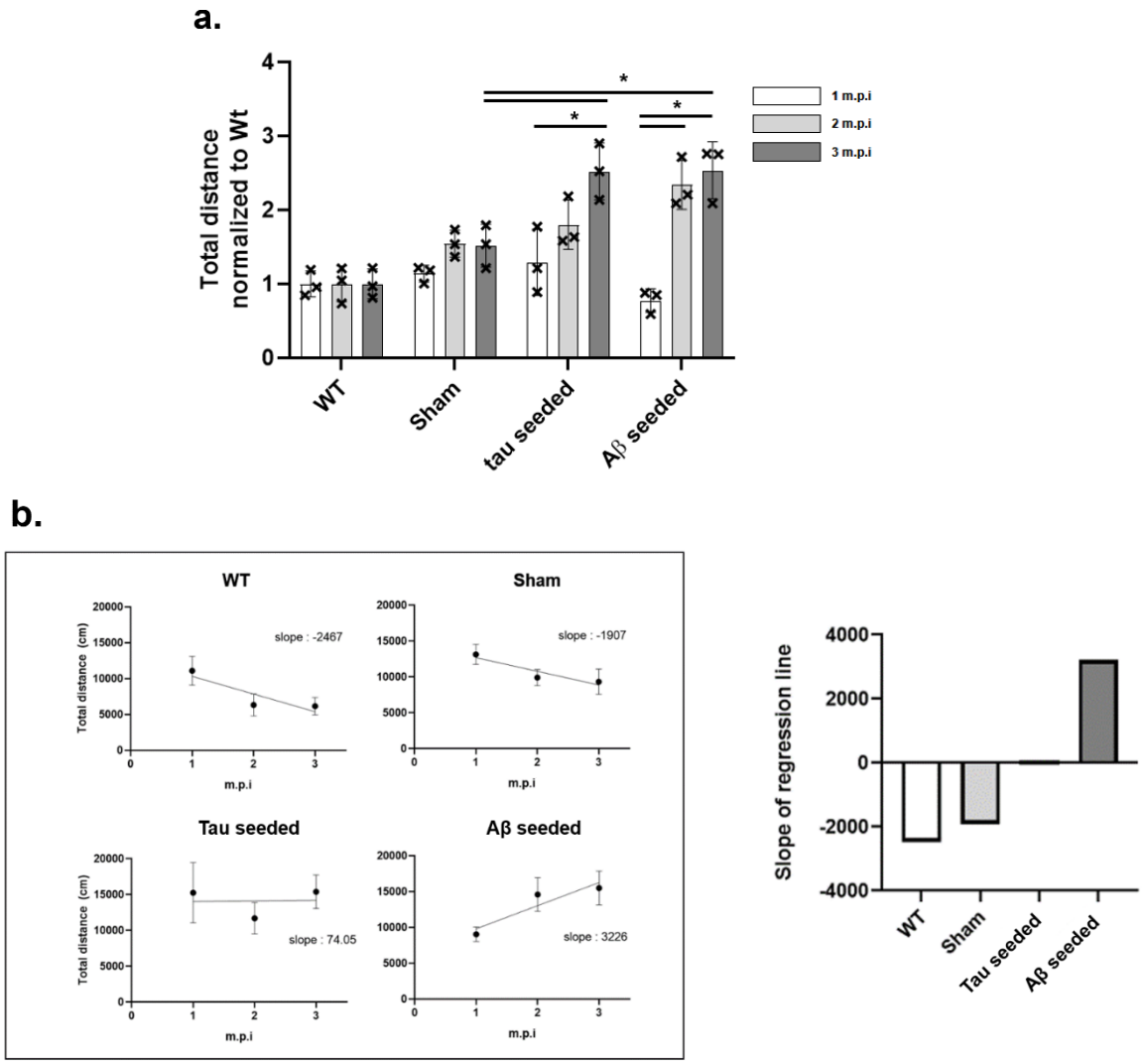


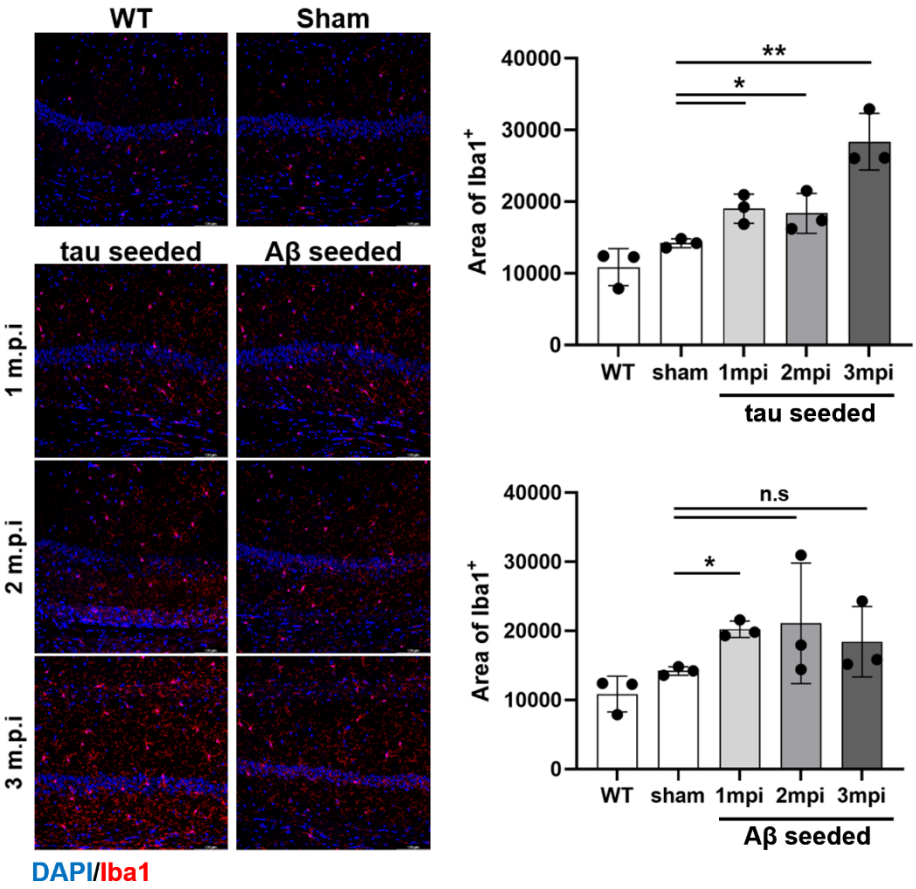
Figure 4. Behavioral abnormality of tau propagation model with hyperactivity

a. Normalized data of total distance (m) to WT values. Data are clustered by group and arranged in order of m.p.i. The incremental total distance was observed in both tau and Aβ seeded group compared to WT and sham. **b.** Regression line of values of total distance was described along months post-injection (left panel). Representative slope of regression line of each group (right panel). Data were expressed as AVE ± SD ($p < 0.05$ (*), $p < 0.005$ (**))

Seeded tau induced microglia activation in hippocampus of P301L mice

Reactive microglia have been considered to play a pivotal roles. However, the patterns of microglial changes with tau pathology and exact mechanism of contribution to tau pathology is remained to defined. In this study, I further examined how microglia changed responsive to seeded tau. I detected significantly more Iba1 positive activated microglia in tau seeded group compared to WT and sham group (Fig. 5). Whereas in Aβ seeded group, altered activation of microglia was not found. And microglial activation was correlated with tau hyperphosphorylation with 0.7762 of correlation coefficient in tau seeded group (Fig. 5b). Importantly, results indicate crucial contribution of microglial activation to tau pathology in tau seeded model.

a.


WT
Sham
tau seeded
Aβ seeded
1 m.p.i
2 m.p.i
3 m.p.i
DAPI/Iba1
Area of Iba1+
40000
30000
20000
10000
0
**
*
WT
sham
1mpi
2mpi
3mpi
tau seeded
n.s
*
Aβ seeded

b.

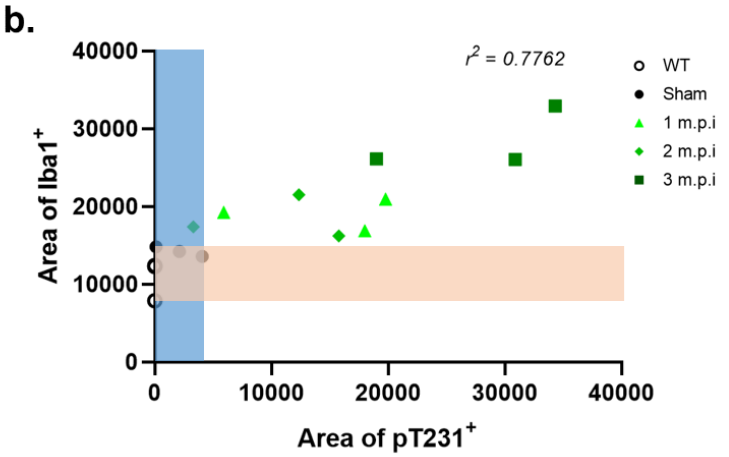

r2 = 0.7762
WT
Sham
1 m.p.i
2 m.p.i
3 m.p.i
Area of Iba1+
Area of pT231+
0
10000
20000
30000
40000

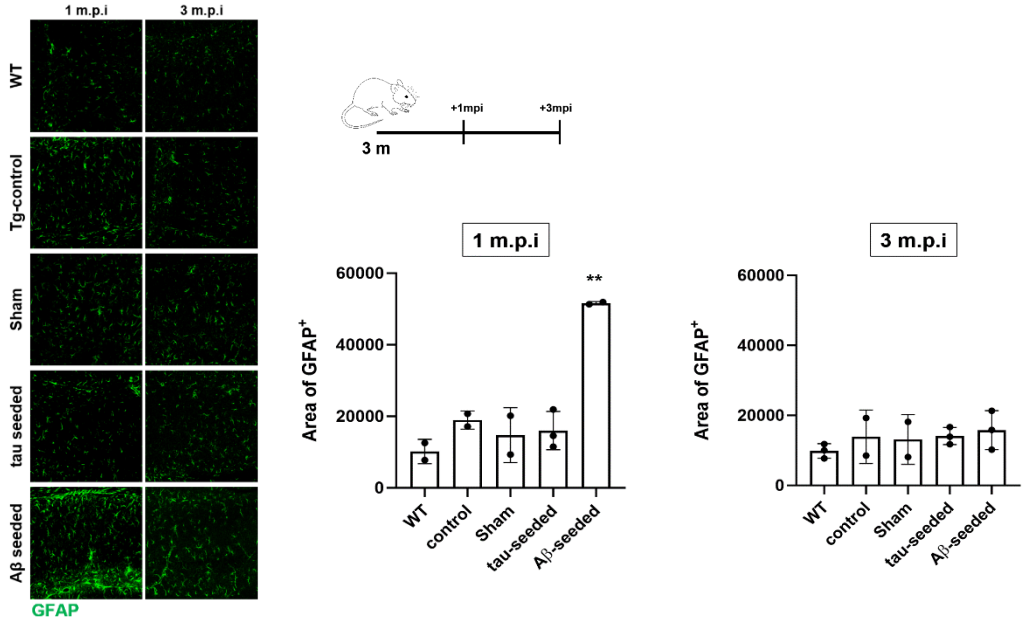
Figure 5. Microglia activation in hippocampus of P301L mice induced by seeded tau

a. Representative merged images of Iba1 positive microglia (Iba1, red) and nucleus (DAPI, blue) immunostaining in hippocampus of WT, sham, tau and Aβ seeded group. Quantification of the area of Iba1 positive microglia of a left panel. **b.** Representive correlationship between $Iba1^+$ microglia and $pT231^+$ between all groups. Data were expressed as AVE±SD ($P < 0.05$ (*), $P<0.005$ (**)).

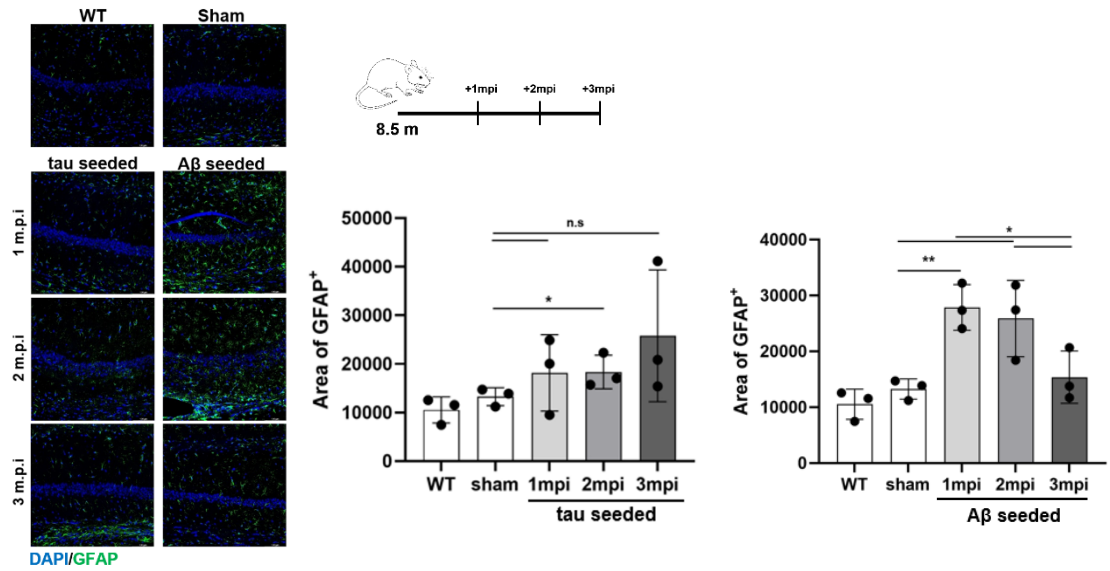
Seeded Aß induced transient activation of astrocytes in hippocampus of P301L mice

To investigate the contribution of astrocytes on tau pathology, reactive astrocytes were immunostained using GFAP antibodies (Fig. 5a-c). Surprisingly, I found that GFAP positive reactive astrocytes was significantly increased in Aβ seeded group at 1 m.p.i (Fig. 5a), and gradually decreased until 3 m.p.i (Fig. 5b). Whereas in tau seeded group, altered activation of astrocytes was not found. And the pattern of astrocytic activation by Aβ is inversely related to tau hyperphosphorlation and hyperactive behavior (Fig. 5c). Therefore, This finding suggests that the that initially activated astrocytes by Aβ would trigger tau pathology and then fink out on it.

a.


1 m.p.i
3 m.p.i
WT
Tg-control
Sham
tau seeded
Aβ seeded
GFAP
+1mpi
+3mpi
3 m
1 m.p.i
3 m.p.i
Area of GFAP+
60000
40000
20000
0
**
WT
control
Sham
tau-seeded
Aβ-seeded

b.


WT
Sham
tau seeded
Aβ seeded
1 m.p.i
2 m.p.i
3 m.p.i
DAPI/GFAP
+1mpi
+2mpi
+3mpi
8.5 m
Area of GFAP+
50000
40000
30000
20000
10000
0
n.s
*
WT
sham
1mpi
2mpi
3mpi
tau seeded
**
Aβ seeded

c.

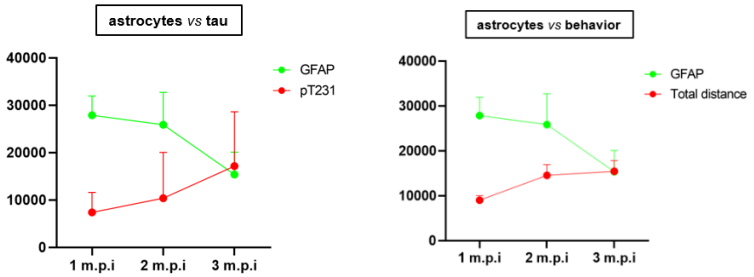

astrocytes vs tau
40000
30000
20000
10000
0
GFAP
pT231
1 m.p.i
2 m.p.i
3 m.p.i
astrocytes vs behavior
GFAP
Total distance

Figure 6. Transient activation of astrocytes in hippocampus of P301L mice induced by seeded Aβ

a, b. Analysis of younger mice injected at 3, 8.5 months of age (a and b respectively). Representative merged images of GFAP positive astrocytes (GFAP, green) and nucleus (DAPI, blue) immunostaining in hippocampus of WT, Tg-control, sham, tau and Aβ seeded group. Quantification of the area of GFAP positive astrocytes. **c.** Representive correlationship between $GFAP^+$ and $pT231^+$ (c left panel) or total distance (c right panel). data were expressed as AVE±SD ($P < 0.05$ (*), $P<0.005$ (**)).

Discussion

Validation of sufficient tau propagation mouse model by injection of tau and Aβ

I generated the rapid tau propagation mouse model by inoculation of lysates from P301L hTau transgenic mice into hippocampus of P301L (Fig. 1a). I found significantly increased tau hyperphosphorylation and propagation to posterior hippocampus up to 3 months compared to WT and sham group (Fig. 3b). Several studies have showed rapid induction of $AT8^{+}$ pathology or NFTs formation by 1~4 weeks post-injection (Ahmed et al., 2014; Iba et al., 2013; Peeraer et al., 2015). Others, however, have shown them 6, 11 months after injection (Clavaguera et al., 2009; Lasagna-Reeves et al., 2012). In tau seeding experiments, pathological outcomes are highly dependent on types and dose of tau source, host mouse and infused region. So, there are mixed evidences about how rapidly tau facilitate the onset of pathology. Nevertheless, tau seeding mouse model is efficient to scrutinize the pathomechanism of tau propagation rather than intact tau transgenic mouse that tardily exhibits its pathology (See Tg-control group of Fig. 3a). Hence, I suggest that the model generated on this study was validated and its pathological outcomes are consistent with other studies.

I also generated the tau propagation mouse model by inoculation of lysates from 5XFAD mice expressing human Aβ into hippocampus of P301L (Fig. 1a). This model exhibits

increased tau hyperphosphorylation and propagation to posterior hippocampus up to 3 months, similarly to tau seeded model compared to WT and sham group (Fig. 3b). Reminiscent of tau seeding experiments, intracerebral inoculation of Aβ have been conducted to establish tau pathologic model (Götz and Chen et al., 2001; Bolmont et al., 2007). Even though there are factors that induce slightly different pathological results in Aβ seeding experiments and there are mixed and controversial evidences, induction of tau pathology is commonly shared features in Aβ seeding on tau transgenic mice. These evidences of Aβ-induced tau pathology imply Aβ-tau interaction on AD progression. But, the relative mechanism of intercorrelate interaction of Aβ and tau is still poorly understood. For many years, amyloid-cascade, which proposes that changes in Aβ promote the disease progression and deleterious cascade such as tau pathology and neurodegeneration, has been prevailing. On the other hand, emerging and accumulating evidences provide compelling focus that tau and Aβ pathologies cooperate with each other for disease process (Pickett et al., 2019; Busche et al., 2019; Busche and Hyman, 2020). Therefore, this finding that seeded Aβ induced tau propagation suggests verification of tau propagation model to scrutinize Aβ and tau interaction and coincidence with outcomes of other studies.

Tau transgenic mice have also typically exhibited a hyperactive phenotype correlated with progression of tau pathology (Jul et al., 2016). And It is known that cooperation with Aβ and tau causes a hyperactivity behavioral phenotype (Pickett et al., 2019). To observe their hyperactive phenotype, I conducted open field test. I found significantly increased hyperactive behavior of both tau and Aβ seeded models in open field for 3 m.p.i compared to WT and sham group (Fig 4), suggesting that not only seeded tau but also Aβ can induce

hyperactive phenotype and two models were sufficiently validated as tau propagation model.

Altered phenotype of glial cells with tau pathology dependent on seeded tau or Aβ

During progression of tau pathology, glial cells such as microglia, astrocytes would be considered to play a pivotal roles. However, there is rarely experimental research which directly determines the differences of progressive pathology and glial activation under the each condition of inoculation with tau and Aβ. Here, I found two compelling findings that; i) seeded tau induced activation of microglia around tau pathology in hippocampus compared to WT and sham group (Fig. 5a) and ii) in Aβ seeded model, levels of $GFAP^+$ reactive astrocytes were drastic increased at 1 m.p.i and then show decremental aspect by 3 m.p.i, inversely related to tau pathology (Fig. 6a, b). There are mixed evidences for this. Ahmed et al provides an evidence that subtle increase in $Iba1^+$ microglia in their tau seeded model is unrelated to the tau pathology and just an early inflammation to neurodegeneration (Ahmed et al., 2014), but is quite weak evidence. Peeraer et al suggested that tau injected mice exhibited $Iba1^+$ microgliosis (Peeraer et al., 2015). Given previously mentioned evidences that depletion of microglia or blockage of NLRP3 inflammasome pathway prevented tau pathology and $CX3CR1^{-/-}$ activated microglia induce tau pathology, activated microglia would be presumed as culprit of tau pathology. Consistent with these evidences, I suggest that increased microglial activation with tau pathology in this study was induced by seeded tau and they could contribute to facilitate tau propagation (Fig. 5). To further scrutinization for this, it is

crucial that alter microglial population and identities in not only injected site but also overall whole brain area due to spatiotemporal heterogeneity of microglia by multi omics analysis (Masuda et al., 2019; Li et al., 2019).

The 5XFAD mice expressing human APP and PSEN1 transgenes, recapitulating major features of AD, show their reactive glial cells by Aβ. To confirm astrocytic changes on Aβ-induced tau pathology excluding already activated astrocytes, the Aβ seeded tau transgenic model would be suitable. I found immediately activated astrocytes with tau hyperphosphorylation in injected site at 1 m.p.i (Fig. 6a). Subsequently, their decremental levels to 3 m.p.i were detected (Fig. 6b). These features was inversely related to tau pathology. Therefore, It suggest that initially activated astrocytes by Aβ would trigger tau pathology and then fink out on it. There is rarely experimental research for astrocytic alteration on tau pathology induced by injected Aβ. Therefore, it is necessary to study of the factors inducting astrocyte contribution to tau pathology and to study the changes population and identities of astrocytes due to their spatiotemporal heterogeneity by multi omics analysis.

Meaning of differences in glial cells changes by tau or Aβ injection

In 2018, the National Institute on Aging and the Alzheimer's Association (NIH-AA) defined a biomarker-based diagnosis of AD to assess progression to A/T/N (amyloid, tau and

neuronal loss) (Jack et al., 2018). ATN staging is confirmed by amyloid-, tau-PET, CSF Aβ 42/Aβ40, CSF total-/p-tau, FDG and MRI, which can understand AD pathological changes but cannot predict the prognosis. In ATN staging, individuals who exhibit changes in amyloid pathology have a high probability of developing tau deposition and cognitive impairment, but not all individuals with amyloid deposition will develop tau deposition and cognitive impairment. And sice tau deposition correlates with cognitive impairment (Betthauser et al., 2020), it is necessary to select a group that develop to tau deposition and cognitive impairment among the AD's continuum that amyloid deposition occurs. Therefore, the two models used in this study were built to evaluate the role of amyloid in the progression of tau pathology of AD. The difference in reactive glial cell by tau and Aβ revealed by the two tau propagation models is evaluated as predictor of prognosis in individuals with amyloid deposition. That is, astrocytes activation by Aβ induced onset of tau deposition, which could provide target information for the development of diagnostic markers or therapeutics. The development of diagnostic markers for reactive astrocytes is expected to screen individuals who will develop tau deposition in the group with amyloid deposition, and therapeutics that control reactive astrocytes may prevent cognitive impairment through inhibition of tau pathology.

Taken together, two tau propagation models by tau or Aβ inoculation have been established and validated to understand tau pathology in AD. These two models showed that phosphorylated tau was propagated from the inoculated area and that tau increased microglia activation and Aβ increased astrocyte activation. These glial cell activation patterns were induced differently by tau and Aβ, respectively, which may mean that tau propagation is

induced differently in AD compared to tauopathy. Therefore, mechanism studies are necessary to understand the characteristics of AD-specific tau pathology, and these characteristics will provide information for understanding the causes of AD and developing prevention and treatments for AD.

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국문 초록

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타우단백질 (tau protein) 과 베타-아밀로이드 (Aβ) 병리는 알츠하이머병 (Alzheimer's disease, AD) 의 특징으로써 병인학적 원인으로 여겨진다. 특히, 신경세포에서 과인산화 (hyperphosphorylation)된 타우 응집이나 세포간 전파 (tau propagation) 와 같은 타우병리는 알츠하이머병에서 신경사멸, 인지기능 저하와 직결되기 때문에 이에 대한 기작연구가 필요하다. 또한, 뇌내 신경세포를 비롯한 미세아교세포 (microglia) 와 별아교세포 (astrocytes) 같은 아교세포들은 병원체나 뇌 손상으로부터 신경을 보호하지만 타우 병리환경에서의 역할은 정확히 밝혀지지 않았다. 따라서 기 연구는, 알츠하이머병에서 타우병리의 특성과 활성 아교세포들의 변화와 역할을 이해하기 위해 타우 병리모델을 확립하고, 타우 병리에 따른 아교세포들의 활성 변화를 관찰함으로, 알츠하이머병과 아밀로이드 독립적인 타우병증 사이의 타우병리 차이점을 확인했다. 병리학적 타우를 발현하는 형질전환쥐 (P301L) 와 아밀로이드를 과발현하는 형질전환쥐 (5XFAD) 의 뇌를 추출하여 각 병리적 타우 또는 아밀로이드를 포함하는 용해물 (lysates) 을 얻었고, 이를 P301L 좌측 해마 (hippocampus) 에 각각 접종한 두 타우 병리모델을 확보했다. 이 두 모델들에서 단기간에 유의미하게 증가한 타우병리를 관찰하였고 더불어 행동실험에서 활동과잉의 행동양상을 확인함으로써 효율적인 타우 병리모델임을 검증하였다. 또한 각 모델에서

구별되게 나타나는 특이적인 아교세포 활성양상을 발견하였다. 타우 접종을 통해 유도된 타우 병리모델은 별아교세포보다 미세아교세포의 활성이 대폭 증가하였으며 이에 반해 아밀로이드 접종한 타우 병리모델은 접종 후 1 개월에 별아교세포의 활성이 대폭 증가하였고 2 개월에 걸쳐 감소하는 양상을 확인하였다. 따라서 기 연구 결과를 통해 아밀로이드 의존적 타우 병리는 기존 타우 병리와 그 기작을 달리 한다는 것과 그 중심에 별아교세포의 역할이 중요하다는 추론을 할 수 있었다. 이러한 동물모델들은 AD 특이적 진단, 치료 연구를 위해 사용될 수 있을 것이다.

핵심어 : 알츠하이머병, 타우단백질, 베타아밀로이드, 활성아교세포

학번 : 2019-23996