ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
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海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

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受 付 番 号　201870008
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（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：ペンシルベニア州　（国名：米国）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
疾患特異的なフィブリルコアに着目したタウ抗体の開発―診断・治療への新規アプローチ

3. 派遣期間：平成　30　年　7　月　1　日　～　令和　2　年　6　月　30　日

4. 受入機関名及び部局名
Center for Neurodegenerative Disease Research, Perelman School of Medicine at the University of Pennsylvania

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

The purpose of my research was to elucidate the mechanism and find the clue to cure tauopathy. Before coming to Center for Neurodegenerative Disease research (CNDR), I was planning to make disease-specific tau antibodies based on the tau fibrils. However, just before I came to CNDR, they succeeded in developing Alzheimer disease (AD)-specific tau antibodies, which were called GT-7 and GT-38 (*Garrett GS et al., JNEN, 2018*). So, I shifted the focus from developing disease-specific tau antibodies to elucidate the pathological mechanisms of tauopathy in more detail.

These days mixed pathology has been getting attention to figure out the nature of neurodegenerative disease, including tauopathy, synucleinopathy and TDP-43 proteinopathy. Patients diagnosed as a certain neurodegenerative disease were often found out to have co-pathologies after being assessed by autopsy (*Robinson JL et al., Brain, 2018*). And the more co-pathology they have, the more severe their symptoms are.

Aging-related tau astrogliopathy (ARTAG) is defined as pathological accumulation of abnormally phosphorylated tau protein in astrocytes. ARTAG is frequently observed as a comorbid pathology in a variety of neurodegenerative diseases including AD, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), frontotemporal lobar degeneration (FTLD), and other synucleinopaties and TDP-43 proteinopaties (*Kovacs GG et al., Acta Neuropathol Comm, 2018*) (Figure 1). Its frequency increases with aging, and cortical ARTAG is associated with dementia in aged patients (*Robinson JL et al, Acta Neuropathol, 2018*).

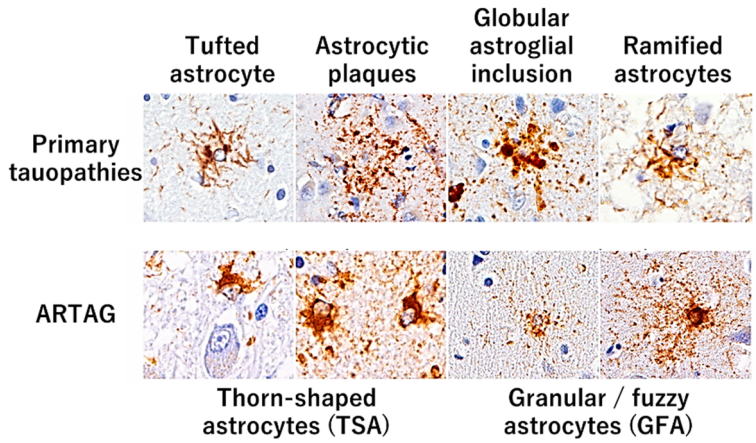

Figure 1. The morphological differences between ARTAG and primary tauopathies. ARTAG contains thorn-shaped astroctyes (TSA) and granular/fuzzy astrocytes (GFA) *(courtesy of Kovacs GG et al., Acta Neuropathol, 2016)*

Recently, the characteristics of ARTAG has partially unveiled: (1) ARTAG is composed of 4-repeat tau and suggested to have distinct conformation, (2) ARTAG has seeding activity and

produces tau pathology in neurons and astrocytes when injected into the mouse brains (*Ferrer I et al. Brain Pathol, 2018*). However, because ARTAG often exists in the vicinity of primary neuronal and glial tauopathies such as neurofibrillary tangle (NFTs), neuropil threads (NTs), and neuritic plaques (NPs) in AD, tufted astrocytes in PSP, and astrocytic plaques in CBC, it's hard to extract pure ARTAG without any contamination of primary tauopathies, which limits detailed analysis of ARTAG.

I have been tackling this problem. I have looked at hundreds of AD cases which have AD pathology (NFTs, NTs, and NPs) in the gray matter and ARTAG in the white matter. I found some cases which had a certain amount of ARTAG in the white matter with enough distance from AD pathology containing gray matter. I selected 4 different regions from one case: (1) the white matter of occipital lobe (Occi-WM) and (2) corpus callosum (CC) for ARTAG-tau extraction, and (3) the gray matter of frontal lobe (Front-GM) and (4) that of cingulate gyrus (Cing-GM) for AD pathology (NFTs, NTs and NPs) (Figure 2).

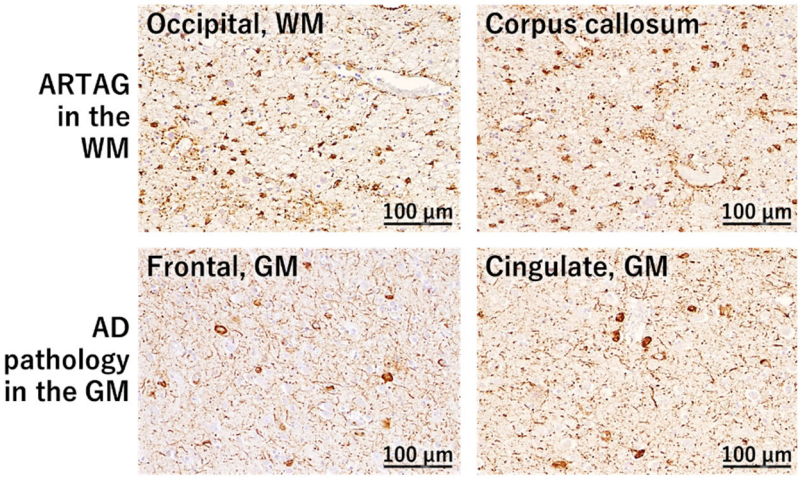

Figure 2. A case of ARTAG for tau extraction (97 y.o., F). This case has abundant ARTAG in the white matter of occipital lobes and corpus callosum, and abundant AD pathology in the gray matter. (PHF1 staining)

I extracted sarkosyl-insoluble tau from each sample according to the already established protocol (*Guo J et al., JEM, 2016*) and assessed the tau in each fraction by western blot and ELISA (Figure 3). The tau concentration of sarkosyl-insoluble tau from occipital-WM and CC was very low and the purity was less than 1/10 of that from Frontal-GM and Cing-GM (The concentration and purity of sarkosyl-insoluble tau: Occi-WM, 7.95 ng/µl and 0.40%; CC, 23.81 ng/µl and 1.02%; Front-GM, 402.67 ng/µl and 9.90%: Cing-GM, 23.81 ng/µl and 9.88%).

When I transduced sarkosyl-insoluble tau from these different regions in the primary neuron and astrocyte co-culture, endogenous tau was aggregated in the neurons. And there were no morphological and distributional differences between AD-PHF (sarkosyl-insoluble tau from Front-GM / Cing-GM) and ARTAG-tau (sarkosyl-insoluble tau from Occi-WM / CC) (Figure 3).

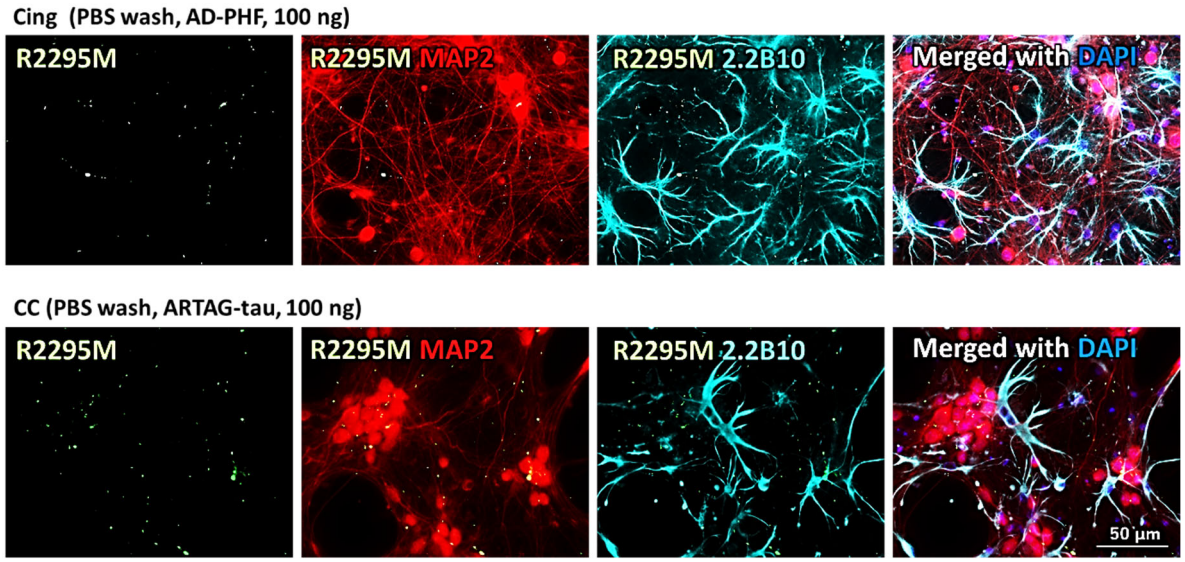

Figure 3. Tau transduction to co-culture of neurons and astrocytes.
Endogenous tau aggregation was induced by both AD-PHF from Cing-GM and ARTAG-tau from CC. Antibodies: R2295M, mouse-tau; MAP2, neuronal marker; 2.2B10, astrocyte marker)

However, because the purity of ARTAG-tau was so low, abundant contaminated toxic proteins made the neurons vulnerable and clustered. (Figure 4)

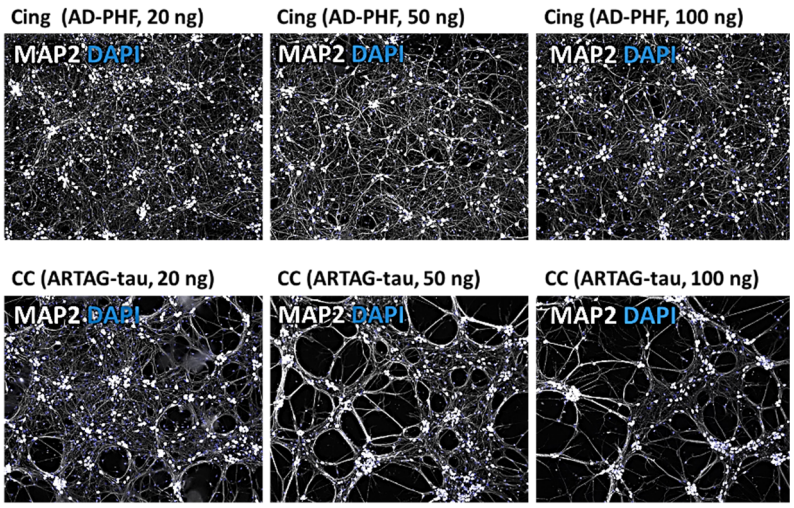

Figure 4. ARTAG-tau from CC was toxic to the neurons.
The neurons became weaken and clustered by transduction of ARTAG-tau from CC in a dose dependent manner.

In the western blot, the band patterns of AD-PHF and ARTAG-tau detected by PHF1 and 17025 showed no difference. However, the band patterns of ARTAG-tau detected by 4R-tau antibody was different from that of AD-PHF, which suggest that the conformation of ARTAG was different from that of NFTs, NTs, and NPs. The ARTAG-tau was also detected by 3R-tau antibody and the band pattern was the same as that of AD-PHF, although the signal intensity was low in the ARTAG-tau (Figure 5).

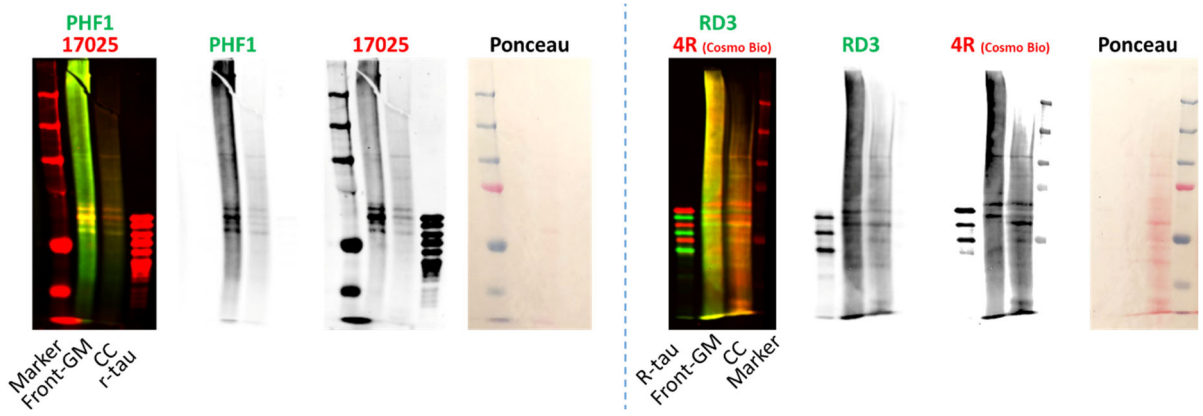

Figure 5. Western blot analysis using AD-PHF (Front-GM) and ARTAG-tau (CC). The band patterns of AD-PHF and ARTAG-tau detected by PHF1 and 17025 were comparable. ARTAG-tau showed different band pattern from AD-PHF by using 4R-tau antibody but also detected by 3R-tau antibody and showed the same band pattern as AD-PHF. Antibodies: PHF1, phosphorylated tau at Ser396/Ser404); 17025, pan-tau; RD3, 3-repeat tau; 4R (Cosmo Bio), 4-repeat tau.

These results suggested two possibility: (1) ARTAG is 4-repeat tau dominant but not exclusively 4-repeat tau. (2) The neurons which contain NFTs in their cell bodies might also have AD-PHF in their axons in the white matter where I extracted ARTAG-tau. I stained ARTAG in this case with 3-repeat and 4-repeat tau antibodies to see whether ARTAG contains not only 4-repeat but also a small amount of 3-repeat tau. The ARTAG was not detected by 3-repeat tau, suggesting ARTAG itself was only composed of 4-repeat tau or 3-repeat tau content was so little that it did not detected by immunohistochemistry. Next, I extracted sarkosyl-insoluble tau form the CC of different AD cases and non-tauopathy cases which have no ARTAG pathology in the white matter. Interestingly, I found small amount of 3-repeat tau was contained in the CC of AD cases but not in the non-tauopathy cases although both cases had no

obvious tau pathology including ARTAG in the CC. I did immunohistochemical analysis using various tau antibodies for the CC of AD cases and found the tiny staining detected by PHF1, AT8 and GT38, an AD-specific antibody. These results suggest axonal tau was pathologically phosphorylated and aggregated in the CC of AD brains.

Although I did not wrap up the data during the 2-year fellowship, I will continue to explore the property of ARTAG-tau by extraction of purer ARTAG-tau, transduction of ARTAG-tau into primary neuron, and injection of ARTAG-tau into mouse brain.