氏　　名	ASLINA BINTI　PAHRUDIN ARROZI
学位の種類	博士（医学）
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学位論文題目	Nasal extracts from patients with Alzheimer’s disease induce aggregates in a cellular model of tau propagation（アルツハイマー病患者由来鼻粘膜抽出物はタウ伝播細胞モデルにおいて凝集体形成を誘導する）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

※整理番号	947	（ふりがな） 氏　名	アスリナ ビンティ パルディン アロズィー Aslina Binti Pahrudin Arrozi
学位論文題目	Nasal extracts from patients with Alzheimer's disease induce aggregates in a cellular model of tau propagation. (アルツハイマー病患者由来鼻粘膜抽出物はタウ伝播細胞モデルにおいて凝集体形成を誘導する)		

Background:

Emerging evidence have shown that tau aggregates can propagate in a hierarchical manner between synaptically connected neurons in the brain, similar to a prion-like propagation. This tau prion activity has been typically studied in brain extracts of patients with Alzheimer's disease (AD). We recently reported a significantly elevated level of phosphorylated tau (p-tau) in nasal smears of patients with AD compared with age-matched, cognitively normal individuals. Therefore, it is likely that tau seeds with the ability to propagate tau aggregation also exist in the nasal tissues of these patients. However, it is difficult to verify this hypothesis using biochemical approaches, such as enzyme-linked immunosorbent assay (ELISA). Conversely, cellular bioassays such as the cellular models of tau propagation would help verify the existence of tau seeds in the nasal cavity of patients with AD.

Purpose:

To investigate the prion seeding activity of tau in nasal mucosa tissues using a cell culture model of tau propagation.

Method:

We developed two cell biosensors; HEK293T cells expressing three repeat (3R) or four-repeat (4R) domains of tau with the L266V, V337M (3RD*VM) and P301L and V377M mutations (4RD*LM) fused to the enhanced green fluorescence protein (EGFP) respectively. This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Shiga University of Medical Science (reference number R2019-102). Postmortem nasal and brain tissues from Pick's disease (PiD) patient, Alzheimer's disease (AD) patients (n=10) and normal subjects (n=10) were obtained from Choju Medical Institute of Fukushimura Hospital, Japan. The tau aggregation in cells was first optimized using brain homogenates from PiD and AD.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

Then, both the brain and nasal homogenates were pre-incubated with Lipofectamine 2000 and added to the cells. The tau aggregate formation was observed after 3 days under inverted fluorescence microscope. The percentage of cells with aggregates was calculated from the image at five different regions for each sample. The level of fluorescence intensity was measured by spectrophotometry. We also measured the level of phosphorylated tau (p-tau) and total tau (t-tau) by ELISA and calculated the p-tau/t-tau ratio. Next, we performed correlation analysis between tau prion activity and the level of tau.

Result and discussion:

The brain tissue homogenates isolated from both AD and PiD induced significant tau aggregation and showed increased fluorescence per cell compared to control L2000 and control brain homogenates in cells expressing tau 3RD*VM-EGFP. However, only brain tissue homogenates from AD patients showed significant tau aggregation and increased in fluorescence per cell compared to control L2000 and control brain homogenates in cells expressing tau 4RD*LM-EGFP. Whereas, the brain and nasal tissue homogenates isolated from patients with AD significantly induced tau aggregate formation in HEK293T cells expressing tau 3RD*VM-EGFP and 4RD*LM-EGFP as seen by the increased fluorescence per cell and percentage of cells with aggregates compared with the brain and nasal tissue homogenates from control samples. By ELISA, the levels of p-tau and t-tau were significantly increased and decreased; therefore, the ratio of p-tau to t-tau (p-tau/t-tau ratio) was significantly increased in the brain tissue homogenates of patients with AD compared with control brain tissue homogenates. In contrast, there was no significant difference in the levels of p-tau and t-tau and in the p-tau/t-tau ratio between the nasal tissue homogenates from patients with AD and controls. The correlation analysis showed that there was no significant association in the level of p-tau, t-tau, or p-tau/t-tau as well as the tau prion activity between the brain tissue homogenates and the nasal tissue homogenates. In addition, in tau 3RD*VM-EGFP and 4RD*LM-EGFP expressing cells seeded with the brain samples, the tau prion activity was strongly positively correlated with the p-tau/t-tau ratio levels in the AD group. No correlation was observed between the tau prion activity and the p-tau/t-tau in cells seeded with the nasal samples.

Conclusion:

These results suggest that the nasal tissues contain tau seeds, similar to the brain, albeit without changes in the levels of p-tau and t-tau. Therefore, a cellular bioassay using nasal tissues would have great potential as an AD biomarker because of the usefulness of nasal tissue biopsy.

別紙様式９（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	947	氏　名	ASLINA BINTI PAHRUNDIN ARROZI
論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

本研究は、アルツハイマー病(AD)患者脳の異常タウ蛋白質が細胞間伝搬するという知見に着目し、異常タウの存在が報告されている鼻腔組織と、蛍光定量モニターが可能な細胞培養株を用いた評価システムを構築し、以下の結果を得た。

1. GFP 標識した 3R あるいは 4R タウ蛋白質を恒常的に発現するヒト不死化細胞 (HEK293T) 株を用いて、凝集体形成効率をモニターする細胞評価系を構築した。
2. 3R タウ蛍光発現在細胞はピック病と AD 患者剖検脳由来の組織ライセートの投与によって、さらに 4R タウ発現細胞は AD 由来のライセートのみで蛍光強度と凝集体形成細胞数の増大を認めたが、ピック病患者では認めなかった。
3. AD 患者の剖検鼻腔採取組織のライセートの投与においても、3R、4R タウ蛋白質の蛍光強度と凝集体形成細胞数の有意な増大を認めた。
4. 脳ライセート投与による培養細胞の蛍光強度や凝集体形成細胞数は、ライセートのリン酸化タウ/総タウ(p-tau/t-tau)比と有意に相関したが、鼻腔ライセートの投与による蛍光強度とは有意な相関を認めなかった。

以上の研究成果は、AD 患者の鼻腔組織が細胞に取り込まれて病的タウ凝集体形成能を有することを示し、その定量的な測定系を確立した重要な研究成果であり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（総字数　598 字）

（令和 3 年 8 月 24 日）