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学位の種類	博士（医学）
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学位論文題目	LC3/FtMt Colocalization Patterns Reveal the Progression of FtMt Accumulation in Nigral Neurons of Patients with Progressive Supranuclear Palsy （LC3/FtMt 共局在パターンは、進行性核上性麻痺患者の黒質ニューロンにおける FtMt 蓄積の進行を明らかにする）
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別紙様式４（課程博士・論文博士共用）

論 文 内 容 要 旨

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博士論文題目	LC3/FtMt Colocalization Patterns Reveal the Progression of FtMt Accumulation in Nigral Neurons of Patients with Progressive Supranuclear Palsy (LC3/FtMt共局在パターンは、進行性核上性麻痺患者の黒質ニューロンにおけるFtMt蓄積の進行を明らかにする)		

Background:
Mitochondrial ferritin (FtMt) is a mitochondrial iron storage protein associated with neurodegenerative diseases. In patients with progressive supranuclear palsy (PSP), FtMt was shown to accumulate in nigral neurons. In neurodegenerative diseases like Alzheimer's, pTau accumulation induces mitochondrial abnormalities and defective mitophagy, resulting in excessive mitochondrial fragmentation. We previously observed the accumulation of pTau in the midbrain of PSP patients colocalizing with FtMt. Using the LC3-IR to stage neuronal damage, we retraced LC3/FtMt patterns and revealed the progression of FtMt accumulation in nigral neurons. Informed by these findings, we proposed a hypothesis to explain the function of FtMt during PSP progression.

Purpose:
To examine FtMt and LC3 in the postmortem midbrain of control and PSP cases to elucidate novel aspects of the associated pathology.

Method:
All tissue samples were obtained from the brain bank at the Shiga University of Medical Science. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Shiga University of Medical Science (reference number R2016-026). Post-mortem midbrains from normal individuals (n = 4) and patients diagnosed with PSP (n = 4) were prepared. For FtMt immunohistochemistry, deparaffinized sections were subjected to heat-induced epitope retrieval (HIER) in 1 mM EDTA. The sections were incubated overnight with C65-2, followed by successive incubations with biotinylated anti-mouse IgM BA 2020 and avidin-biotin-peroxidase complex (ABC). For LC3 immunohistochemistry, HIER was performed just after deparaffinization in 10 mM citrate buffer (pH 6.3). The sections were then incubated with rabbit polyclonal against LC3, followed by incubation with HistostarTM (Ms + Rb) before revelation with DAB. Counterstaining was performed via immersion in hematoxylin. For the double immunofluorescence histochemistry, sections were incubated in Alexa-Fluor-555-labeled donkey anti-mouse IgG and Alexa-Fluor-488-labeled goat anti-rabbit IgG.

（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で印字すること。
２．※印の欄には記入しないこと。

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True black solution was applied to reduce endogenous fluorescence. Digital images were acquired using a Leica TCS SP8 equipped with a Leica DMi8 microscope. Colocalization of FtMt and LC3 were then characterized using IMARIS and further processed with MeshLab.

Result and discussion:

For single immunostaining of SNcs of the control cases, weak-to-moderate FtMt immunoreactivity (FtMt-IR) was preferentially observed in neurons. Weak LC3 immunoreactivity (LC3-IR) was preferentially found in the SNc neurons. In the SCs of the control cases, there was no apparent FtMt-IR. Faint LC3-IR was anecdotally observed in a few neurons. In the SNcs of the PSP cases, strong-to-intense FtMt-IR was observed. Strong LC3-IR was observed in most neurons. As in the control cases, a few neurons with faint LC3-IR were observed in the SC. The SC of a PSP case was devoid of FtMt-IR. As in the control cases, a few neurons with faint LC3-IR were observed in the SC. The SC of PSP patients anecdotally showed a few neurons with faint LC3-IR. For double immunofluorescence of SNcs of the control cases, weak-to-moderate FtMt-IR and LC3-IR were localized in the soma of neurons. In the SNcs of the PSP cases, intense FtMt-IR and LC3-IR were also localized in the soma of neurons. Quantitative analysis of fluorescence signal intensities showed a significant 2.5-fold FtMt-IR increase ($p = 0.028$) and a significant threefold LC3-IR increase ($p = 0.029$) in the SNcs of the PSP patients. Correlation analysis for the FtMt-IR and LC3-IR intensities in the control and PSP cases' SNcs showed a value higher than 0.73, indicating either a tendency for the FtMt-IR and LC3-IR to increase concomitantly in all neurons or subpopulations of neurons following different trends. Recurrent FtMt-IR and LC3-IR patterns were investigated to identify possible neuronal subpopulations. Four colocalization patterns were characterized based on the feature observed under 3D imaging.

Conclusion:

In the nigral neurons of the PSP patients, concomitant accumulation of LC3/FtMt seemed to be related to mitophagy processes. Using the LC3-IR to stage the progression of neuron damage, we resolved the FtMt-IR progression in the PSP patients. This staging could help to elucidate pathological processes in PSP and to understand the role and function of FtMt in PSP.

別紙様式９（課程博士・論文博士共用）

博士論文審査の結果の要旨

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（博士論文審査の結果の要旨）

本研究は、進行性核上性麻痺（PSP）患者脳の黒質ニューロンのFtMtの発現と局在に着目して、神経細胞障害の指標としてオートファゴソームマーカーであるLC3との免疫染色を、PSP4症例と非神経疾患4症例の剖検脳で実施し、以下の結果を得た。

1. 正常対照の中脳黒質ニューロンではFtMtおよびLC3のシグナルは軽度であった。中脳上丘のニューロンではFtMtおよびLC3のシグナルはほとんど確認できなかった。
2. PSPの中脳黒質ニューロンでは、強く集積したFtMtのシグナルを認め、LC3の発現もほとんどのニューロンで強く認めた。中脳上丘のニューロンでは、FtMt、LC3の発現は弱かった。
3. FtMtとLC3の蛍光二重染色の結果から、どちらの発現局在はニューロンの細胞体であり正常対照に比べてPSPでは有意に強くシグナルを認め、症例におけるFtMtとLC3のシグナルの強さには正の相関があった。
4. 3D画像解析によるFtMtとLC3の局在パターンから4つの類型に分類が可能であった。PSPにおけるFtMt蓄積の程度の指標になり、マイトファジーの関与を示唆するものと考えられた。

以上の研究成果は、PSPの病理におけるFtMt蓄積の意義を考える上で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。（597字）

（令和5年1月25日）