Characterization of lysosomal proteins Progranulin and Prosaposin and their interactions in Alzheimer's disease and aged brains: increased levels correlate with neuropathology.

doi: 10.1186/s40478-019-0862-8(https://doi.org/10.1186/s40478-019-0862-8)

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学位の種類	博士（医学）
学位記番号	博士甲第872号
学位授与の要件	学位規則第４条第１項
学位授与年月日	令和２年　９月９日
学位論文題目	Characterization of lysosomal proteins Progranulin and Prosaposin and their interactions in Alzheimer’s disease and Aged Brains:Increased Levels Correlate with Neuropathology （リソソームタンパク質のプログラニュリンとプロサポニンの性質とアルツハイマー病および加齢脳における相互作用：神経病理所見と比例して増加する）
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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

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学位論文題目	Characterization of lysosomal proteins Progranulin and Prosaposin and their interactions in Alzheimer's disease and Aged Brains: Increased Levels Correlate with Neuropathology (リソソームタンパク質のプログラニュリンとプロサポニンの性質とアルツハイマー病および加齢脳における相互作用：神経病理所見と比例して増加する)		

Background

Alzheimer's disease (AD) is the leading cause of dementia in the worldwide. To date, in spite of promising experimental data in AD animal models, therapies to prevent or remove Aβ have generally had limited effects in clinical trials to slow down cognitive decline, other approaches are also needed. Progranulin (PGRN) is a protein encoded by the GRN gene with multiple identified functions including as a neurotrophic factor, tumorigenic growth factor, anti-inflammatory cytokine and regulator of lysosomal function. A single mutation in the human GRN gene resulting in reduced PGRN expression causes types of frontotemporal lobar degeneration resulting in frontotemporal dementia. Prosaposin (PSAP) is also a multifunctional neuroprotective secreted protein and regulator of lysosomal function. Interactions of PGRN and PSAP affect their functional properties. As a result of the previous reports of increased PGRN expression in AD brains in contrast to the deficits occurring in FTD due to GRN mutations, detailed investigations are needed.

Purpose

Characterizing the features of Progranulin and associated protein in the progression of Alzheimer's disease pathology in human brain samples

Method

We examined in detail the cellular expression of PGRN and PSAP, and their association with Alzheimer's pathology in middle temporal gyrus samples of a series of human brain cases (n=45) staged for increasing plaque pathology by enzyme based and fluorescent immunohistochemistry. Relative protein expressions were assessed by Western blotting in same series of samples. Interaction of PGRN and PSAP in human brain samples were revealed by co-immunoprecipitation, and colocalizations were confirmed with confocal images.

（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。

Result and discussion

Immunohistochemistry showed PGRN expression in cortical neurons, microglia, cerebral vessels and amyloid beta (Aβ) plaques, while PSAP expression was mainly detected in neurons and Aβ plaques, and to a limited extent in astrocytes. We showed that there were increased levels of PGRN protein in AD cases and corresponding increased levels of PSAP. Levels of PGRN and PSAP protein positively correlated with Aβ, with PGRN levels correlating with phosphorylated tau levels in these samples. Although PGRN colocalized with lysosomal associated membrane protein-1 in neurons, most PGRN associated with Ab plaques did not. Aβ plaques with PGRN and PSAP deposits were identified in the low plaque non-demented cases suggesting this was an early event in plaque formation. We did not observe PGRN positive neurofibrillary tangles. Co-immunoprecipitation studies of PGRN from brain samples identified only PSAP associated with PGRN, not other known PGRN binding proteins, under conditions used. Most PGRN associated with Aβ plaques were immunoreactive for PSAP showing a high degree of colocalization of these proteins that did not change between disease groups. As PGRN supplementation has been considered as a therapeutic approach for AD, the possible involvement of PGRN and PSAP interactions in AD pathology needs to be further considered.

Summary

From these findings, it can be concluded that the presence of neuroprotective molecules PGRN and PSAP on Aβ plaques do not prevent the formation of these structures. Significant interactions between these molecules were demonstrated biochemically, and by immunohistochemical techniques in neurons and also associated with Aβ plaques. Secreted forms of PGRN have multiple protective and anti-inflammatory properties, but these might not be evident when this protein is deposited with PSAP around Aβ plaques. Increased total protein levels of PGRN and PSAP was evident in AD samples but not those with less pathology, but the presence of PGRN and PSAP on most plaques in low pathology control cases with fewer smaller plaques indicate this is an earlier event in development of AD pathology. Binding of these proteins on Aβ plaques might have ameliorating effects of Aβ toxicity on surrounding cells, or it might be hindering the removal of Aβ plaque by infiltrated microglia.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

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（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

アルツハイマー病は認知症の第一の原因である。リソソームタンパク質のプログラニュリンはGRN遺伝子によってコードされるが、その発現を減少させるヒトでの変異は、前頭側頭葉変性症を起こすことが知られていた。プロサポシンは神経保護作用がある分泌性因子であり、プログラニュリンとの相互作用で機能変化が起こることが知られていた。先行研究ではアルツハイマー病患者の剖検脳でプログラニュリンの発現亢進が観察されていたが、本論文では、以下の点を明らかにした。

1）プログラニュリンは皮質神経細胞など広く発現が見られた一方、プロサポシンは神経細胞とAβプラークでのみ見られた。
2）プログラニュリンとプロサポシンの発現は、ADが進行するにつれてAβプラークに占める割合が上昇した。
3）プログラニュリンとプロサポシンは特異的に結合する。
4）プログラニュリンとプロサポシンはAβプラークにおいて共局在する。
5）プログラニュリンはマイクログリア由来のリソソームと相関する。

以上より、本論文は、アルツハイマー病剖検脳でのプログラニュリンとプロサポシンの発現について新たな知見を得たものであり、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５４４字）

（令和２年８月２４日）