博 士 論 文 の 要 旨 及 び 審 査 結 果 の 要 旨

氏　　名	ANISIMOV Sergei
学　　位	博士（医学）
学 位 記 番 号	新大院博（医）　第 901 号
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博 士 論 文 名	G3BP1 inhibits ubiquitinated protein aggregations induced by p62 and USP10 （G3BP1 は p62 と USP10 によるユビキチン化蛋白質の凝集体形成を抑制する ）
論文審査委員	主査　教授　小野寺　理 副査　教授　神吉　智丈 副査　講師　渡辺　啓介

博士論文の要旨

Background

The aberrant accumulation of ubiquitinated protein aggregates in cells is a common cause of many degenerative diseases, such as α-synuclein in Parkinson's disease (PD) and CFTR-ΔF508 in cystic fibrosis (CF). Ubiquitin-specific protease 10 (USP10) and a ubiquitin receptor p62 have been shown to augment ubiquitinated protein aggregation. USP10 interacts with p62 and augments p62-induced ubiquitinated protein aggregation. Ras GTPase-activating protein-binding protein 1 (G3BP1) and G3BP2 are binding proteins of USP10. In this study, we examined whether G3BP1 or G3BP2 are involved in regulation of ubiquitinated protein aggregations by USP10 and p62.

Methods and Results

We reduced the expressions of G3BP1 and G3BP2 proteins in HeLa cells (G3BP1-knockdown [KD], G3BP2-KD) using small interfering RNA. G3BP1-KD increased the amount of ubiquitinated proteins in HeLa cells, and the increase was attenuated by a treatment with a proteasome inhibitor MG-132. In contrast, G3BP2-KD did not show stimulatory activity on the amount of ubiquitinated protein.

CFTR-ΔF508 is a ubiquitination- and aggregation-prone mutant protein and a causative factor of CF. G3BP1-KD in HeLa cells increased the amount of CFTR-ΔF508, and the increase was attenuated by G3BP2-KD. Immunostaining experiment showed that CFTR-ΔF508 aggregation was augmented by G3BP1-KD. While G3BP2-KD hardly affected CFTR-ΔF508

aggregate formation, it reduced the G3BP1-KD-induced aggregate formation.

We examined whether USP10 and/or p62 plays a role in G3BP1-KD-induced protein aggregations. G3BP1-KD increased the amounts of CFTR-ΔF508 and ubiquitinated proteins, and these increases were reduced by both USP10-KD and p62-KD. Immunofluorescence staining showed that G3BP1-KD increased the amount of CFTR-positive aggregate formation, and this increase was diminished by both USP10-KD and p62-KD. In addition, USP10 overexpression augmented the G3BP1-KD-induced increase in CFTR-ΔF508 protein. Furthermore, the amount of exogenous α-synuclein in HeLa cells was increased by G3BP1-KD, and the increase was reduced by both USP10-KD and p62-KD. An immunoprecipitation analysis showed that both G3BP1 and G3BP2 interact with p62 and USP10.

A ubiquitination assay with His-tagged ubiquitin showed that G3BP1-KD in HeLa cells increases the amounts of ubiquitinated α-synuclein and CFTR-ΔF508.

Cell viability assay showed that G3BP1-KD little affected the cell viability with CFTR-ΔF508 expression, but G3BP-1-KD together with USP10-KD or p62-KD markedly reduced the cell viability induced by CFTR-ΔF508.

The brain tissue of both PD patients and non-PD individuals expressed a relatively low amount of G3BP1 to those of USP10, p62 and G3BP2.

Discussion

In this study, we found that G3BP1 inhibits ubiquitinated protein aggregation by reducing the amount of ubiquitinated proteins in the steady state of cells, and these activities target two pathogenic proteins: CFTR-ΔF508 and α-synuclein. Therefore, the present study suggested that the reduction of G3BP1 and/or its dysfunction promotes pathological ubiquitinated protein aggregation in degenerative disorders, including CF and PD.

G3BP1 depletion stimulated p62/USP10-induced protein aggregation, and the stimulation was attenuated by G3BP2 depletion. Both G3BP1 and G3BP2 interacted with p62 and USP10. Taken together, these results suggested that G3BP1 inhibits p62/USP10-induced protein aggregation, whereas G3BP2 attenuates such a G3BP1 inhibition by competing G3BP1-interaction with p62 and/or USP10.

Evidence suggests that while ubiquitinated protein oligomers have cell toxicity, such toxicity is attenuated by forming big ubiquitinated protein aggregates. The present findings support this notion, as follows. Ubiquitinated CFTR-ΔF508 by itself did not show cell toxicity, since ubiquitinated CFTR-ΔF508 is rapidly degraded by proteasome. G3BP1-KD increased the amount of ubiquitinated CFTR-ΔF508 but not the toxicity, since G3BP1-KD simultaneously induced

CFTR-ΔF508 aggregation. However, p62-KD or USP10-KD in G3BP1-KD cells restored CFTR-ΔF508 toxicity by reducing CFTR-ΔF508 aggregation.

We detected a low amount of G3BP1 protein relative to G3BP2, p62 and USP10 in PD and non-PD brains. It should be noted that G3BP1-knockout mice develop neurodegeneration with neuronal dysfunction and neuronal apoptosis. These results suggest that despite its low expression in brains, G3BP1 still plays a protective role in the neuronal survival and development of neurodegeneration.

審査結果の要旨

パーキンソン病(PD)は、脳の黒質のドーパミン神経細胞の変性と脱落をきたす進行性の神経変性疾患である。PD 患者脳には、ユビキチン化したα-シヌクレイン蛋白質の凝集体が共通して認められ、α-シヌクレインは、PD の原因蛋白質と考えられている。α-シヌクレイン蛋白質は、高熱、酸化剤などのストレスに曝されると、ユビキチン化され、神経細胞毒性を示す。

申請者は、多くのユビキチン化蛋白質にて、そのユビキチン化と凝集体形成を抑制する分子として、G3BP1 を同定した。さらに、G3BP1 の標的蛋白質の１つとして、α-シヌクレインを同定した。

培養細胞株において、G3BP1 の発現の低下により、α-シヌクレインのユビキチン化が著明に昂進し、α-シヌクレインの蛋白量が増加することを見出した。

また、USP10 蛋白質と p62 蛋白質は、α-シヌクレインの凝集体形成を誘導することが報告されている。申請者は、USP10 と p62 のダブルノックダウン実験により、この時のユビキチン化蛋白質凝集体の誘導を、G3BP1 が抑制していることを見出した。さらに、PD および正常人の脳(amygdala)にて、G3BP1 の発現が他の組織よりも低いことを示した。この事から、ユビキチン化蛋白質の凝集体形成が、脳では起こりやすいことが示唆された。

本研究は、G3BP1 が、ユビキチン化蛋白質の量を減少させ、凝集体形成を抑制する分子であることを示した。また、G3BP1 が、ユビキチン化α-シヌクレインの凝集体形成を低下させることを細胞系で示し、患者脳の結果と合わせて、G3BP1 が PD の発症を抑制している可能性を示した。これらを明らかにした点に、博士論文としての価値を認めた。