博 士 論 文 の 要 旨 及 び 審 査 結 果 の 要 旨

氏　　名	PIATNITSKAIA Svetlana
学　　位	博士（医学）
学 位 記 番 号	新大院博（医）　第 902 号
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博 士 論 文 名	USP10 is a critical factor for Tau-positive stress granule formation in neuronal cells （ USP10 は Tau 陽性のストレス顆粒の形成に重要な役割を果たす ）
論文審査委員	主査　教授　神吉　智丈 副査　教授　小野寺　理 副査　准教授　中津　史

博士論文の要旨

Background

Tau is a microtubule-associated protein implicated as the causative factor of several neurodegenerative diseases, including Alzheimer's disease (AD). AD is a progressive neurodegenerative disease that impairs memory and other mental functions and it is the most frequent cause of dementia. Tau aggregates in neurons of brain lesions are a hallmark pathology of AD. Accumulating evidence suggests that stress granules (SGs) initiate Tau aggregation in cultured cells and AD. SGs are stress-inducible aggregates containing RNA-binding proteins and RNAs that exert protective activities against various stresses. Under certain stress conditions, Tau is localized in SGs in cultured cells. Ubiquitin-specific protease 10 (USP10) is a component of SGs, and it promotes SG formation in various stress conditions. We examined whether USP10 plays a role in Tau-positive SG formation in neuronal cells.

Methods and Results

HT22 is a mouse hippocampal neuronal cell line. MG-132 is a proteasome inhibitor that has been shown to induce SG formation. MG-132-treatment of HT22 cells induced multiple Tau aggregates in the cytoplasm, and numerous Tau-aggregates were colocalized with four SG marker proteins (USP10, TIA1 G3BP1 and PABP). Like HT22 cells, MG-132 treatment of primary neuron-rich cells for 8 h induced Tau/TIA1/USP10-triple-positive SGs. However, USP10-depletion in HT22 cells by short hairpin USP10-RNA (USP10-KD) severely attenuated Tau/TIA1-positive SGs. TIA1 has been

shown to induce Tau-positive SG formation. Overexpression of green fluorescent protein (GFP)-tagged TIA1 (GFP-TIA1) in HT22 cells induced Tau-positive SG formation, but the formation was severely reduced by USP10-KD. These results suggested that Tau is localized in SG with USP10 and TIA1 in HT22 cells, and the formation of Tau-positive SGs requires USP10 protein.

The overexpression of HA-tagged USP10 (HA-USP10) without MG-132 treatment in HT22 cells induced Tau/TIA1-SG formation, and these SGs were colocalized with HA-USP10. USP10 has a deubiquitinase activity for several substrates, and $USP10^{C424A}$ is the deubiquitination defective mutant. $USP10^{C424A}$ induced Tau-positive SGs with an activity equivalent to that of wild type USP10 ($USP10^{WT}$), indicating that deubiquitinase activity of USP10 is dispensable for Tau/TIA1/USP10-SG formation. $USP10^{1-274}$ and $USP10^{275-798}$ were C- and N-terminal USP10 deletion mutants, respectively. Both $USP10^{1-274}$ and $USP10^{275-798}$ induced Tau-positive SGs at levels equivalent to that of $USP10^{WT}$.

Mutations and phosphorylation of Tau (pTau) are associated with Tau aggregation in AD. pTau-positive inclusions in brain lesions are a hallmark pathology of AD. The co-expression of USP10 with Tau in non-neuronal cells (HeLa) increased the amounts of Tau and pTau.

Immunohistochemical staining detected pTau-positive inclusions in cell bodies and neurites of the neurons of AD brains. USP10 was predominantly detected in cell bodies of most neurons and some USP10 in cell bodies are colocalized with pTau inclusions.

Discussion

Recent evidence has shown that TIA1-induced Tau-positive SG formation plays a critical role in Tau aggregation and Tau neurotoxicity in AD pathogenesis. In the present study, we found that USP10 plays a key role in Tau/TIA1/USP10-SG formation in cultured neuronal cells. Furthermore, USP10 was colocalized with pTau-positive inclusions in brain lesion of AD. Therefore, the present study suggested that USP10 and TIA1 cooperatively promote pTau aggregation in AD through SG formation.

USP10 mutants revealed that the deubiquitinase activity of USP10 is dispensable for the SG-inducing activity and that both N- and C-terminal regions of USP10 ($USP10^{1-274}$ and $USP10^{275-798}$) have separate SG-inducing activities. We previously showed that both $USP10^{1-274}$ and $USP10^{275-798}$ fragments independently interact with the ubiquitin receptor p62[25]. Given that Tau is a ubiquitinated protein, these results suggest that p62 might play a role in the USP10-induced Tau-positive SG formation.

Tau is a microtubule binding protein. The overexpression of USP10 increased the amount of pTau. The binding of Tau to microtubules is attenuated by the phosphorylation of Tau at multiple sites. Thus, pTau is more easily released from microtubules than non-phosphorylated Tau and is efficiently recruited into SG. pTau increased by USP10 might therefore play a role in USP10-induced Tau-positive SG formation.

審査結果の要旨

アルツハイマー病（AD）は認知症を引き起こす最も頻度が高い疾患である。タウ蛋白質はADの原因蛋白質である。AD患者の脳病変には、リン酸化したタウ蛋白質の異常な凝集体が観察される。このタウ凝集体は神経細胞に毒性を示し、ADの発症に深く関与している。一方で、ストレスに曝された神経細胞において、タウ蛋白質がストレス顆粒に局在し、異常な凝集体を形成し、神経毒性を示すことが報告されている。しかしながら、タウ陽性のストレス顆粒の形成機構については不明な点が多い。

申請者は、ubiquitin specific protease 10(USP10)蛋白質がタウ陽性のストレス顆粒の形成を誘導することを発見した。培養神経細胞をプロテアソーム阻害剤で処理すると、タウ陽性のストレス顆粒が誘導された。このストレス顆粒の形成は、USP10 の発現を低下させると著明に低下した。さらに、神経細胞株にUSP10を過剰発現させるだけでも、タウ陽性のストレス顆粒の形成が誘導された。このストレス顆粒の誘導活性には、USP10 の脱ユビキチン化活性は不要であった。AD 患者の脳病変において、USP10 はリン酸化したタウ蛋白質の凝集体と共局在した。

本研究は、USP10 が、神経細胞においてタウ蛋白質の凝集体形成を誘導することを示した。さらに、USP10 が AD 患者のタウ凝集体の形成に関与することを示唆した。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。