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学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（薬科学）　氏 名 胡 安琪

学 位 論 文 題 名

Mechanism of altered cleavage of substrates by γ-secretase including presenilin without pathogenic gene mutations
（遺伝子変異に依存しないγ－セクレターゼによる基質の切断変化機構の解明）

Introduction

The production, aggregation, and accumulation of amyloid β-protein (Aβ) in the brain are initial steps in the pathogenesis of Alzheimer's disease (AD). Aβ is generated from amyloid precursor protein (APP) which is cleaved first by β-secretase, then cleaved by γ-secretase at multiple γ-sites and generates different kinds of Aβ products. The dysfunction of γ-secretase which may correlate to increased neurotoxic Aβ42 generation is consider as a cause to initiate AD. Familial AD (FAD) harboring *PSEN* gene mutations generates more neurotoxic Aβ42, whereas it is controversial whether sporadic AD (SAD) increases Aβ generation. Previous research of our lab showed evidence that some SAD may involve γ-secretase dysfunction, causing aberrant cleavage of multiple γ-secretase substrates. However, the mechanisms(s) underlying these altered cleavages of substrates in the absence of causative mutation of *PSEN* genes remain uncovered. Cleavages of substrates by γ-secretase occur in membrane microdomains called lipid rafts, which are enriched in cholesterol. Thus, we suppose that altered cholesterol level may influence γ-secretase cleavages of APP and/or other γ-secretase substrates. To demonstrate this hypothesis, we assayed γ-secretase ability to produce Aβ in lipid rafts of brain samples and cultured cells with *in vitro* γ-secretase assay.

Results and discussion

lipid raft can be isolated biochemically as a detergent CHAPSO-resistant membrane (DRM). We prepared DRM from brain samples of sporadic AD and age-matched non-demented subjects. We analyzed microlocalization of γ-secretase component, PS1 CTF. We found that the recoveries of PS1 CTF from DRM decreased significantly in AD subjects compared to non-demented controls, indicating the active γ-secretase complex including PS1 endoproteolytic fragments tended to disperse from DRM in AD subjects. We then examined the cholesterol levels in DRM samples of AD and non-demented subjects. We found that the DRM cholesterol level of SAD subjects significantly lower than the non-demented controls. We next examined Aβ generation with the C99/CTFβ-FLAG substrate by *in vitro* γ-secretase assay. Both Aβ40 and Aβ42 generation significantly elevated in DRM of AD relative to non-demented controls. Ratio of Aβ42/Aβ40 was also higher in AD than in non-demented

controls. Taken together, human brain analysis suggests a hypothesis that the lowered cholesterol contents in brain membrane microdomain DRM of AD subjects.

To assess our hypothesis, we constructed an assay system with DRM of cells with regulated membrane cholesterol level. HEK293 cells were treated with methyl-β-cyclodextrin (MβCD) to withdraw cholesterol from cells, and water-soluble cholesterol (cholesterol-saturated MβCD) to supply cholesterol into cells, and DRM fractions were prepared from these cells. As expectedly, the γ-secretase components dispersed from DRM into non-DRM of cells treated with MβCD, and the recovery of these components into DRM significantly downed compared to non-treated cells. This resembles a feature of DRM derived from brain membrane of AD subjects. In contrast, cells treated with cholesterol-saturated MβCD increased the recovery of the γ-secretase components into DRM significantly, suggesting that the cholesterol level of DRM regulates the residence of γ-secretase complex in DRM. As like as human brain samples, we examined DRM ability in Aβ generation using *in vitro* γ-secretase assay with the C99/CTFβ-FLAG substrate. Aβ40, Aβ42 and Aβ38 production were significantly increased in DRM of cells treated with MβCD which coincides with the result with DRM of human brain samples, while decreased in DRM of cells treated with cholesterol-saturated MβCD. Ratios of Aβ42/40 and Aβ42/38 were not changed among respective DRM fraction. Qualitative changes of Aβ generation may be due to another factor except for cholesterol level in brain DRM.

To explore the possible reason causing the reduction of cholesterol level and changes of γ-secretase activity in DRM of SAD patients' brain, we examined two AD risk factors, aging and diet, with mouse model. However, we failed to find significant change in cholesterol level, microlocalization of γ-secretase components, or Aβ generation in DRM from brain of aging mice or mice fed with low-fat or high-fat diet compared to control. To further confirm the influence of cholesterol level to alter the γ-secretase activity in DRM, we changed the cholesterol level of membrane prepared from mouse brain by treatment with MβCD or cholesterol-saturated MβCD. Although the alteration of microlocalization of γ-secretase components were not observed, we observed significant increase of Aβ42 level in DRM of membrane treated with MβCD, while significant decrease of Aβ40 and Aβ38 in DRM of membrane treated with cholesterol-saturated MβCD. Although these data were not exactly the same as cell experiment, these results confirm that Aβ production is influenced by the cholesterol level in DRM.

Conclusion

Although we cannot rule out other factors that induce the alteration of Aβ generation in sporadic AD, current results suggest that upregulated production of Aβ observed in the dispersion of active γ-secretase component from DRM by lowering the cholesterol level in DRM may be a potent cause of sporadic AD pathogenesis.