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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）ミクログリアに発現するカテプシンＥのAβ分解活性と歯周病菌による発現制御の解析

研究課題名（英文）The enzymatic activity of Cathepsin E in Abeta degradation

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研究成果の概要（和文）：リソソームから漏出したカテプシンBの細胞質での働きがミクログリアによる脳炎症に加えミトコンドリア由来酸化ストレスを増大させ、老化に伴う記憶機能低下（脳老化）の要因となることを示す結果を得た。そこで本研究ではカテプシンBの脳炎症促進因子としての概念を深化させ。本研究により脳老化ならびにアルツハイマー病の脳病態におけるミクログリアにおけるカテプシンBの役割を明確にし、アルツハイマー病予防・治療薬の新規標的分子として確立したい。

研究成果の学術的意義や社会的意義
As the population ages and lifespan increases, the worldwide aging has become a sever society issue. Our study provides potential targets for slowing aging which will contribute to the healthy and slowed aging society.

研究成果の概要（英文）：In this study, we found that genetic ablation of cathepsin B (CatB) in mice significantly reduced the generation of reactive oxygen species (ROS) and neuroinflammation and improved cognitive impairment during aging. Pharmacological inhibition of CatB significantly reduced the generation of mitochondria-derived ROS and proinflammatory mediators induced by L-leucyl-L-leucine methyl ester (LLOMe). In the CatB over-expressing microglia after treatment with LLOMe, which mimicked the aged microglia, CatB leaked in the cytosol is responsible for the degradation of the mitochondrial transcription factor A (TFAM), resulting in the increased generation of mitochondria-derived ROS and proinflammatory mediators through impaired mtDNA biosynthesis. These results suggest that the increase and leakage of CatB in microglia during aging are responsible for the increased generation of mitochondria-derived ROS and proinflammatory mediators, culminating in memory impairment.

研究分野：口腔機能分子科学

キーワード：cathepsin B　lysosomal leakage　microglia　mitochondria

１．研究開始当初の背景

It is widely believed that oxidative stress and inflammation are major causative factors for the progressive decline in motor and cognitive functions that occur during normal aging in humans and animals. The activation of microglia is the main cellular source of oxidation products and proinflammatory mediators in the brain. Cathepsin B (CatB, EC 3.4.22.1), a typical cysteine lysosomal protease, is associated with inflammatory responses by microglia through the production of IL-1β. Furthermore, CatB is a potential molecular switch that shifts microglia/macrophages toward the neurotoxic phenotype through autophagic activation of nuclear factor-κB (NF-κB). More recently, CatB has been demonstrated to play a critical role in neuroinflammation and impairment of learning and memory induced by chronic systemic exposure to lipopolysaccharide derived from *Porphyromonas gingivalis*, the major periodontal bacteria, in middle-aged mice. However, how oxidative stress and chronic neuroinflammation arise during aging remains unclear.

２．研究の目的

The present study aims to clarify the molecular mechanism of CatB induced oxidative stress and inflammation during aging.

３．研究の方法

(1) In vivo, memory behavior tests, LTP and spine density were examined in WT and *CatB*$^{-/-}$ mice of both young (2-month old) and aged (20-month old) groups.

(2) In vivo, oxidation and inflammation were examined in WT and *CatB*$^{-/-}$ mice of both young (2-month old) and aged (20-month old) groups.

(3) In vitro, LLOMe and rotenone were treated in microglia cells to mimic the aged microglia. The inflammatory response and oxidative stress were examined by RT-PCR, Western blotting, Immunofluorescent staining and flow cytometry.

(4) In vitro, the leakage of CatB and enzymatic activity were examined using acridine orange and Z-Arg-Arg-cresyl violet in alive microglia cells. The degradation of pre-TFAM by CatB were analyzed using in vitro digestion assay.

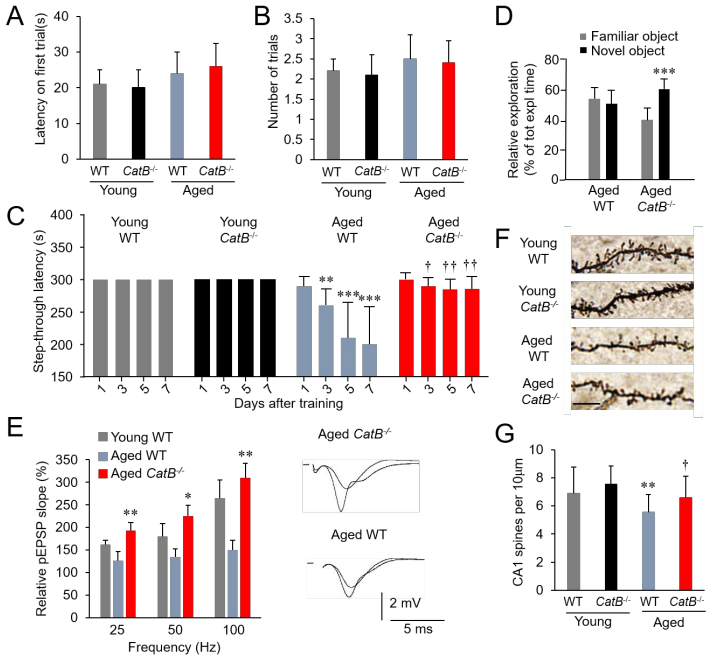
(5) In vivo, memory behavior tests including Y-maze and objective recognition tests were conducted in middle-aged mice by intra-lateral ventricle injection of CatB-overexpressing microglia.

４．研究成果

(1) Amelioration of age-dependent decline in learning and memory of *CatB*$^{-/-}$ mice. There was no significant difference in the latency in the first trial or in the number of trials among all groups (Fig. 1A, B). The retention latencies of both aged groups were significantly longer than those in the acquisition trial. The retention latency of four consecutive trials was significantly longer in aged *CatB*$^{-/-}$ mice than in aged WT mice (Fig. 1C). The effects of CatB deficiency on the age-dependent cognitive impairment were further examined using the novel object recognition test, commonly used simple tests for the hippocampus-dependent learning and memory. Aged WT mice did not

show a response and could not discern a change in the object. In contrast, aged *CatB*$^{-/-}$ mice showed a response to the novel object and were able to discern a change in the object (Fig. 1D).

Fig. 1

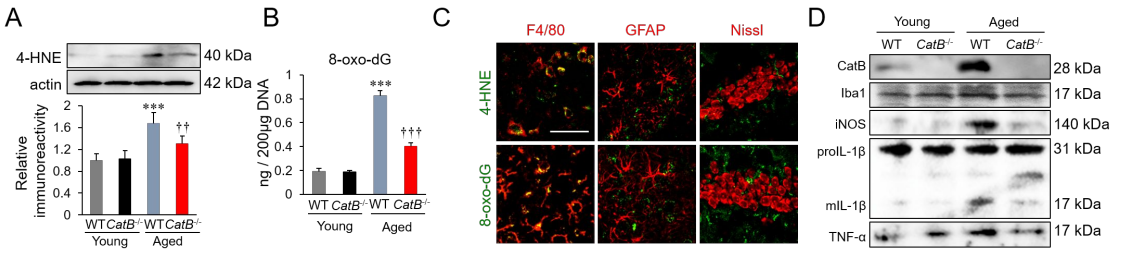


Relative fEPSP slope was measured at 30 min after tetanic stimulation, a significant cumulative potentiation was observed in the hippocampus of young WT mice. In contrast, cumulative potentiation was not observed at 30 min even after tetanic stimulation with 100 Hz in the hippocampus of aged WT mice (Fig. 1E). On the other hand, the mean values of relative fEPSP slope measured at 30 min after stimulation with 25, 50 and 100 Hz in aged CatB-/- mice were significantly larger than those in aged WT mice (Fig.1E).

We further examined the dendritic spine density of CA1 neurons by Golgi-Cox staining. The mean dendritic spine density of CA1 neurons in the aged *CatB*$^{-/-}$ mice was significantly larger than that in the aged WT mice (Fig. 1F, G).

(2) Amelioration of age-dependent increase in oxidation and inflammation in the hippocampus of cathepsin B deficient (*CatB*$^{-/-}$) mice. The mean amounts of these oxidative markers were significantly larger in the hippocampus of aged WT mice than in younger animals (Fig. 2A, B). The mean relative amount of 8-oxo-dG and 4-HNE was significantly lower in aged *CatB*$^{-/-}$ mice than in aged WT mice. To identify the possible cellular origin of oxidative stress, double immunohistochemical staining was conducted. In the hippocampus of aged WT mice, the immunoreactivities of both 8-oxo-dG and 4-HNE were found exclusively in microglia with activated morphology, but not in astrocytes or neurons (Fig. 2C). The expression of inflammatory markers were significantly than those in aged WT mice (Fig. 2D).

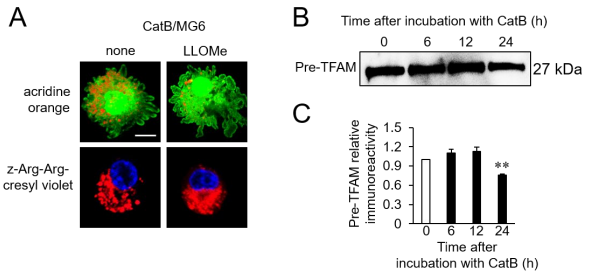
Fig. 2



(3) Possible role of CatB leaked into the cytosol in the degradation of pre-TFAM. The punctate acridine orange aggregates were observed in non-treated control CatB/MG6 cells (Fig.3A). The

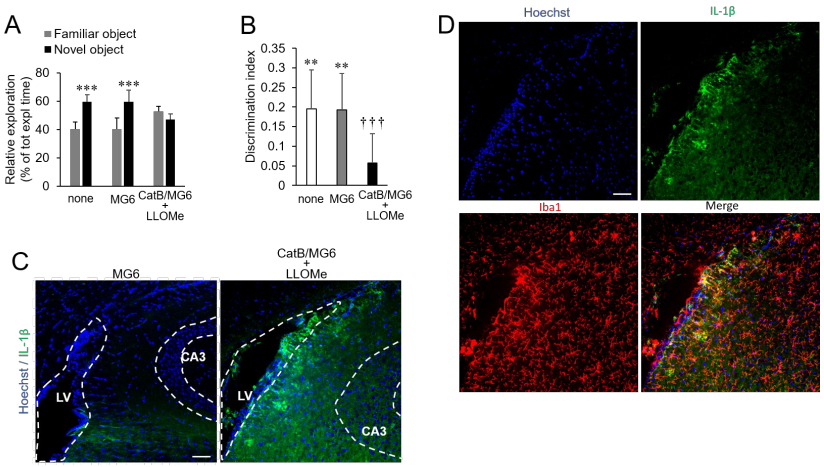
enzymatic activity of CatB was also visible as punctate bright signals in CatB/MG6 cells. On the other hand, LLOMe markedly reduced the fluorescent signal for acridine orange (Fig. 3A). Rather surprisingly, the enzymatic activity of CatB was still remained as diffuse bright signals in CatB/MG6 cells after treatment with LLOMe, suggesting that CatB leaked from lysosomes retained the enzymatic activity even in the cytosol. In *in vitro* digestion assay, human recombinant pre-TFAM was significantly decreased 24 h after incubation with CatB in cleavage buffer at neutral pH, 37°C (Fig. 3B, C).

Fig. 3



(4) Impairment of learning and memory in middle-aged mice by intra-lateral ventricle injection of CatB/MG6 cells. Middle-aged mice subjected to the intra-lateral ventricle injection of culture medium or MG6 cells showed a response to the novel object and were able to discern a change in the object, the middle-aged mice subjected to the intra-lateral ventricle injection of LLOMe-treated CatB/MG6 cells did not show a response and could not discern a change in the object (Fig. 4A, B). At the site of injection, large double positive cells for Iba1 and mIL-1β that were considered to be injected LLOMe-treated CatB/MG6 cells attached to ependymal cells along the lateral ventricle extended their processes to the stratum oriens of the hippocampal CA3 subfield (Fig. 4C, D).

Fig.4



(5) In the present study, we have provided evidence that CatB enhances oxidative stress and inflammatory response in microglia by proteolytic degradation of TFAM after leakage into the cytosol. This observation is first to clarify the critical role of CatB in microglia mediated oxidation and inflammation during aging. In the future, specific microglial CatB knockout mice should be generated to further clarify the function of CatB in microglia in aging and Alzheimer's disease.

５．主な発表論文等

〔雑誌論文〕（計　2　件）

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〔図書〕（計　0　件）

〔産業財産権〕
○出願状況（計　0　件）

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番号：
出願年：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

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