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学位の種類	博　士　（医　学）
学位記番号	博　士　甲第７２０号
学位授与の要件	学位規則第４条第１項該当
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学位論文題目	The FAM3 superfamily member ILEI ameliorates Alzheimer's disease-like pathology by destabilizing the penultimate amyloid-ß precursor （FAM3 スーパーファミリーメンバーILEI はアミロイドβの直前の前駆体を不安定化することによりアルツハイマー病様病理を軽減させる）
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別紙様式３

論 文 内 容 要 旨

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学位論文題目	The FAM3 superfamily member ILEI ameliorates Alzheimer's disease-like pathology by destabilizing the penultimate amyloid-β precursor (FAM3 スーパーファミリーメンバーILEI はアミロイドβの直前の前駆体を不安定化することによりアルツハイマー病様病理を軽減させる)		

Aim of the study:

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. Deposition of aggregated amyloid-β (Aβ) in the brain, caused by persistent imbalance between production and clearance, is central to the pathogenesis of AD, and reduction of brain Aβ is the goal for disease-modifying therapies. Carboxyl-terminal fragments (CTFs) of amyloid precursor protein (APP) are cleaved by the γ-secretase complex to release Aβ. Therefore, the γ-secretase complex, which primarily contains presenilin, nicastrin, APH-1 and PEN-2, is a major target for therapeutic intervention. Besides APP-CTFs, γ-secretase cleaves many other transmembrane proteins including Notch, which is essential to pivotal physiological functions. Application of non-selective γ-secretase inhibitors has caused serious adverse effects due to blockade of Notch signaling and accumulation of neurotoxic APP-CTFs. To overcome these limitations, novel therapeutic strategies are needed. In this study, we aim to search for novel proteins which are able to reduce Aβ generation through interaction with the γ-secretase complex without inhibiting γ-secretase activity.

Methods:

1. Identification of a novel γ-secretase binding protein ILEI: We employed tandem affinity-tag purification (TAP) followed by liquid chromatography-tandem mass spectrometric analysis. Co-immunoprecipitation (co-IP) and chemical cross-linking were used to confirm protein–protein interactions. 2-dimensional (2D) Blue Native (BN) SDS-PAGE was used to analyze protein complex.
2. Analysis of ILEI effects on Aβ metabolism *in vitro*: ILEI expression level was manipulated by transfection with cDNA or siRNA. Recombinant ILEI was purified from conditioned medium of cultured cells. ELISA and Western blotting were used to evaluate ILEI functions in cultured cells.
3. Examination of ILEI expression *in vivo*: ILEI expression in brains of wild-type C57/B6J mice was assessed by immunohistochemistry (IHC). Western blotting and ELISA were used to analyze extracts from organotypic brain slice cultures. Western blotting was used to analyze human autopsy brains.
4. Analysis of ILEI functions *in vivo*: Neuron-specific ILEI-overexpressing transgenic (ILEI-Tg) mice were generated. Double transgenic (DT) mice were obtained by crossbreeding ILEI-Tg and APP-Tg mice, which had been widely used as AD model mice. Mice of each genotype were characterized by Western blotting, IHC, ELISA and Y-maze test.

（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で印字すること。
２．※印の欄には記入しないこと。

Results:

1. ILEI binds to the γ-secretase complex. One of the identified peptides derived from the isolated γ-secretase complex matched residues 164-179 of human ILEI. Co-IP and 2D-BN SDS-PAGE indicated interaction between ILEI and the γ-secretase complex. *In situ* chemical crosslinking followed by co-IP demonstrated that ILEI directly binds with presenilin 1-CTF.

2. ILEI reduces Aβ generation by destabilizing APP-CTFs. Knockdown of endogenous ILEI increased Aβ secretion, while overexpression of ILEI decreased Aβ secretion in cultured cells. However, ILEI knockdown did not affect Notch cleavages or γ-secretase activity. Then, we observed that ILEI knockdown resulted in accumulation of APP-CTFs through extending their half-lives. CTFs of LRP and N-cadherin, other substrates of γ-secretase, were unaffected by ILEI knockdown, suggesting that ILEI specifically destabilizes APP-CTFs.

3. ILEI extracellularly works through interfering substrate-stabilizing capability of presenilin 1. In cultured cells, treatments with recombinant ILEI reduced both intracellular APP-CTFs and secreted Aβ in a dose-dependent manner, which indicated that ILEI might function extracellularly. Recombinant ILEI was endocytosed into cultured cells and associated with intracellular γ-secretase complexes. ILEI's functions depended neither on γ-secretase activity nor competition with γ-secretase substrates, but on its binding with presenilin. ILEI and APP-CTFs did not share a similar binding domain of presenilin.

4. ILEI exhibits neuron-specific expression in mouse and human brains. ILEI was widely expressed in neurons of the central nervous system. TGF-β treatments decreased Aβ generation through up-regulating protein levels of ILEI in cultured rat brain slices. Levels of secreted ILEI decreased dramatically in brains of AD patients compared to age-match control or non-AD neurological diseases patients.

5. ILEI ameliorates the phenotypes of AD model mice. Compared to wild-type mice, ILEI-Tg mice showed decrease in APP-CTFs and Aβ and unaltered Notch cleavages in brains. In DT mice, deficit of the spatial working memory in the Y maze test was rescued to a wild-type control level. Compared to APP-Tg mice, Aβ load in brains of DT mice decreased dramatically as accessed by IHC and ELISA.

Discussion:

In this study, we identify a novel secretory protein ILEI, which regulates the metabolism of APP-CTFs by perturbing substrate-stabilizing capability of presenilin without altering γ-secretase activity. This unique mechanism allows ILEI to reduce Aβ and neurotoxic APP-CTFs at the same time without interrupting other crucial physiological events mediated by γ-secretase activity. Also, the amount of ILEI in human autopsy brains was negatively correlated with the amount of Aβ or phosphorylated tau, indicating a strong correlation between ILEI and AD pathology. Most importantly, expression of ILEI in AD model mice rescued both memory deficits and amyloid deposition, suggesting considerable potential for clinical use in future.

Conclusion:

ILEI is a novel therapeutic target for designing disease modifying therapies of AD.

別紙様式８（課程・論文博士共用）

学位論文審査の結果の要旨

整理番号	７２６	氏　名	刘　磊
論文審査委員			

（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。）

アルツハイマー病患者脳に見られる病理所見として、Ａβタンパク質の蓄積が挙げられ、Ａβ産生を抑制する物質は新たな治療薬の候補となりうる。アミロイド前駆体タンパク質(APP)からAβ産生の鍵を握るγセクレターゼと相互作用するタンパク質として、ILEI を同定し、その性質について詳細な解析を加えた。

その結果、以下の点を明らかにした。

１．ILEI が、γセクレターゼ複合体の１つであるプレセニリンのＣ端と結合し、シャペロン機能を修飾する。
２．ILEI が、APP からβセクレターゼ切断を受けたC端断片を不安定化させることで、その後γセクレターゼによって産生されるＡβ量を低下させる。
３．APP 同様にγセクレターゼの基質である Notch の切断に対しては、効果を有さない。
４．神経細胞特異的に ILEI を発現するトランスジェニックマウスを作製し、アルツハイマー病モデルマウスと交配して解析することにより、ILEI の過剰発現が老齢アルツハイマー病モデルマウスにおけるＡβ蓄積を低下させ、その結果として記憶の障害を軽減させることを見出した。

本論文は、Ａβ産生量を調節する新規タンパク質 ILEI について新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

（総字数５５８字）

（平成２６年９月１日　）