Form 3

# Abstract of Thesis

| Name ( Sabharwal Lavannya ) | |
|---|---|
| Title | The role of Presenilin-1 (Psen1) as a scaffold protein in the NF-κB mediated inflammation<br>(NF-κB を介した炎症におけるプレセニリン1の足場タンパク質としての役割 ) |


## Abstract of Thesis

Chronic inflammation is critical for the development of various diseases. We previously discovered one mechanism associated with this development and specific to nonimmune cells, such as synovial cells, fibroblasts, and endothelial cells, is an NF-κB activator - the inflammation amplifier (formerly IL-6 amplifier), which is activated by a simultaneous stimulation of NF-κB and STAT3 to express inflammatory mediators including chemokines, cytokines and growth factors, which deregulate local homeostasis via an accumulation of various immune cells and proliferate various regional cells that contribute to the development of various inflammatory diseases. The amplifier activation has been observed in several disease models such as F759 arthritis model and EAE as well as in patient samples.
To further understand the detailed molecular mechanism of the inflammation amplifier and its role in human diseases, genome wide screening was performed using 16000 mouse genes and identified 1289 genes that are positive regulators of the synergistic activation of NF-κB. Out of the 1289 genes, I selected Presenilin-I (Psen-1) and investigated its role in detail.

In 2016, we reported that NF-κB-mediated inflammation caused by breakpoint cluster region (BCR) is dependent on α subunit of casein kinase II (CK2α). BCR is the cause of certain types of leukemia upon fusing to Abl tyrosine kinase resulting in abnormal cell survival and proliferation. CK2 is a serine/threonine kinase composed of two catalytic α subunits and two regulatory β subunits. CK2 has been known to play a role in various cellular processes such as cell cycle control, DNA repair, regulation of the circadian rhythm. It was reported that CK2 phosphorylates p65, an action critical for NF-κB-mediated transcription.
In the current study, I demonstrate that Psen1, which is a catalytic component of the γ-secretase complex and the mutations of which are known to cause familial Alzheimer disease (AD), acts as a scaffold for the BCR-CK2α-p65 complex to induce NF-κB activation. Psen1 deficiency in mouse endothelial cells showed a significant reduction of NF-κB p65 recruitment to target gene promoters.

By contrast, Psen1 overexpression enhanced reporter activation under NF-κB responsive elements and IL-6 promoter. Furthermore, the transcription of NF-κB target genes was not inhibited by a γ-secretase inhibitor, suggesting that Psen1 regulates NF-κB activation independently of γ-secretase activity. Mechanistically, Psen1 associated with the BCR-CK2α complex, that phosphorylated p65 at serine 529 and created p300 binding site which increased p65-mediated transcription followed by inflammation development. Consistently, TNF-α-induced phosphorylation of p65 at serine 529 as well as p300 binding was significantly decreased in Psen1-deficient cells. Additionally, the BCR-CK2α-p65 complex association was perturbed in the absence of Psen1.

Therefore, these results suggested that Psen1 functions as a scaffold of the BCR-CK2α-p65 complex and that this signaling cascade could be a novel therapeutic target for various chronic inflammatory conditions, including those in Alzheimer's Disease.

様式 7

# 論文審査の結果の要旨及び担当者

| 氏　名 | （ Sabharwal Lavannya ） | |
|---|---|---|
| | （職） | 氏　名 |
| 論文審査担当者 | 主 査　教授 | 石井　優 |
| | 副 査　教授 | 長澤　丘司 |
| | 副 査　教授 | 仲野　徹 |
| | 副 査　教授 | 山下 俊英 |

## 論文審査の結果の要旨

本研究では、非免疫細胞において転写因子NFκBおよびSTAT3の同時活性化にて生じるケモカイン過剰産生機構として知られる炎症回路の制御遺伝子の1つであるプレセニリン-1の作用機序について検討した。プレセニリン-1は、タンパク分解酵素であるγセクレターゼ複合体の活性因子でありアルツハイマー病の原因遺伝子であるが、本研究では、プレセニリン-1がNFκBシグナル伝達を正に制御する詳細を明らかにした。

申請者は、非免疫細胞の一種である血管内皮細胞などを用いて、RNA干渉法によってプレセニリン-1の発現を阻害した。その結果、炎症回路による転写因子NFκBの標的遺伝子の発現増加が抑制され、その分子機構として、NFκBのこれら標的遺伝子プロモーター領域へ結合が抑制されていた。また、これとは反対に、プレセニリン-1を過剰発現させると、NFκB依存性の転写活性が増強された。これまで知られているプレセニリン-1の機能は、全てγセクレターゼ活性を媒介されている。しかし、NFκB活性化増強はγセクレターゼ阻害剤の添加でも抑制されなかった。そのため、プレセニリン-1によるNFκB活性化増強機構は、新規機能の可能性であることが示唆された。機能解析の結果、プレセニリン-1は、細胞膜にてBreakpoint cluster region (BCR)-カゼインキナーゼ2α複合体と会合することで、NFκBが標的遺伝子に結合するために重要な529番目のセリン残基のリン酸化を促すことが判明した。さらに、プレセニリン-1欠損細胞では、BCR-カゼインキナーゼ2α-NFκB複合体形成が障害されていた。これらの結果は、プレセニリン-1がγセクレターゼ非依存的にBCR-カゼインキナーゼ2α-NFκB複合体の足場タンパク質として働くことが示された。

申請者は、プレセニリン-1の新機能を明らかにし、プレセニリン-1-BCR-カゼインキナーゼ2α-NFκBというシグナル伝達経路が炎症疾患の新規治療標的となる可能性を示した。そのため、本研究は、学位に値するものと認める。