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学 位 論 文 題 名 (Dissertation Title)
Investigating the role of ECRG4 in Alzheimer's disease pathology and diagnosis
(アルツハイマー病の病理と診断におけるECRG4の役割の調査)

【Background and Objectives】

Alzheimer's disease (AD) is a subtype of dementia associated with progressive neurodegeneration, leading to impairments in memory, thinking, and behavior. As a global health crisis, AD is a leading cause of morbidity and mortality among the elderly. The disease is characterized by specific pathological hallmarks, including the formation of cytotoxic amyloid- β ($A\beta$) plaques, intracellular neurofibrillary tangles of hyperphosphorylated tau protein, and neurovascular dysfunction. The accumulation of $A\beta$ is particularly regarded as a critical driver of AD, underscoring the importance of understanding the molecular mechanisms behind $A\beta$ plaque formation. Therapeutic antibodies (Abs) designed to target and clear these plaques have been approved by the FDA. However, these Abs are primarily effective for patients with mild cognitive impairment (MCI) or early-stage AD (EAD), highlighting the need for improved diagnostic tools that can identify MCI and EAD cases with convenience and sensitivity.

Esophageal cancer-related gene 4 (ECRG4), also known as AUGURIN, is a gene located on chromosome 2. Initially identified as a tumor suppressor, ECRG4 is significantly downregulated in malignant tumors, primarily due to promoter DNA methylation. Studies have shown that ECRG4 overexpression inhibits cell proliferation and migration, though its molecular mechanisms remain to be fully elucidated. Additionally, ECRG4 induces the production of pro-inflammatory cytokines, such as IL-6 and TNF- α , in macrophages and microglia, and may play a role in brain aging by inhibiting neural stem cell proliferation. Elevated ECRG4 levels have also been detected in AD model mice and AD patients, suggesting its potential as a novel diagnostic marker for AD.

Current AD diagnostic methods include $A\beta$ positron emission tomography, cerebrospinal fluid (CSF) $A\beta$ measurements, and mass spectrometry-based plasma $A\beta$ detection. However, these methods either require specialized equipment or are highly invasive. There is thus a need for simpler, less invasive diagnostic techniques. ECRG4 is known to be secreted from cells via proteolytic cleavage by enzymes such as thrombin. My research aims to develop a new enzyme-linked immunosorbent assay (ELISA) system to detect ECRG4 in plasma, evaluating its potential as a novel biomarker for diagnosing AD and investigating its possible role in AD pathogenesis.

【Materials and Methods】

To develop an ELISA system for ECRG4 detection in body fluids, both monoclonal and polyclonal Abs against ECRG4 were utilized. RNA sequencing (RNA-seq) was performed on ECRG4-positive samples to analyze gene expression, with validation by RT-qPCR. The GEO database was consulted to identify cell types potentially

influenced by ECRG4. *In vitro* assays, including the MTT assay and immunocytochemistry, evaluated ECRG4's effects on these cells. Additionally, *in vivo* immunohistochemistry was conducted to investigate the impact of intracerebral ECRG4 injection on mouse brain tissue, as well as cellular changes in AD patient brains at various disease stages.

【Results】

We developed an ELISA system for detecting ECRG4, employing the highly sensitive monoclonal antibodies 2A8 as the capture antibody and the polyclonal antibody OB1246 as the detection antibody. This system revealed that ECRG4 is highly expressed in the plasma of AD, MCI, and dementia patients. Analysis of plasma samples with elevated ECRG4 expression suggested a potential association with altered gene expression in oligodendrocyte (OLG) lineage cells in the brain. *In vitro*, ECRG4 promoted apoptosis in OLGs, while *in vivo*, ECRG4 injection into mouse brains led to a decrease in OLG markers, increased oligodendrocyte progenitor cell (OPC) proliferation, perivascular accumulation, and vascular rupture. Additionally, OPC perivascular accumulation was observed in EAD brains, with decreased astrocyte markers in AD brains.

【Discussion】

This study developed a sensitive and specific ELISA system for ECRG4 detection, demonstrating a marked elevation of ECRG4 in plasma from AD, dementia, and MCI patients, with no significant changes observed in CSF. RNA-seq data indicated a close association between ECRG4 expression and OLG lineage cells. *In vitro* and *in vivo* analyses suggested that ECRG4 may contribute to AD pathogenesis by promoting inflammation, OLG apoptosis, OPC dysfunction, and blood-brain barrier (BBB) compromise. Observations in human brains revealed OPC accumulation around blood vessels in EAD stages, with BBB disruption occurring later.

Despite these promising results, our study has limitations, such as a relatively small sample size and the ELISA system's inability to differentiate ECRG4 isoforms. Additionally, ECRG4 demonstrated slightly lower sensitivity and specificity compared to traditional AD biomarkers. Nonetheless, ECRG4 may reflect pathological BBB changes, offering unique insights into OLG apoptosis, BBB integrity, and cerebrovascular pathology—areas less covered by conventional biomarkers. Further validation with larger, more diverse cohorts is needed to confirm ECRG4's diagnostic potential. If validated, ECRG4 could become a non-invasive plasma biomarker and therapeutic target for AD. Moreover, exploring ECRG4's role across different AD stages and its synergy with other biomarkers could deepen our understanding of AD's complex pathology.

【Conclusion】

In conclusion, our study indicates that elevated ECRG4 levels in plasma may serve as a biomarker for dementia symptoms and the ECRG4 may play a critical role in the pathophysiological processes of AD by promoting OLG apoptosis and compromising the integrity of the BBB. Future research should further investigate the specific role of ECRG4 in the pathogenesis of dementia and AD, as well as its potential as a diagnostic biomarker.