Department of Virology

Kazuhiro Kondo, *Professor*　　Nobuyuki Kobayashi, *Assistant Professor*

General Summary

Human herpesviruses (HHVs) are capable of establishing lifelong latent infections of their hosts and are frequently reactivated. We are studying the molecular mechanism of latency and pathogenesis of human cytomegalovirus (HCMV) and HHV-6, and have found a novel latent protein of HHV-6 associated with chronic fatigue syndrome and mood disorders. Additionally, we are attempting to apply HHV-6 and HHV-7 as tools to study the mechanism of fatigue. The amounts of salivary HHV-6 and HHV-7 DNA increased with training and decreased with rest, suggesting the usefulness of these types of DNA as biomarkers of physiological fatigue. Additionally, we study cognitive impairment and Alzheimer's disease (AD), which we have previously shown the relationship to fatigue and HHV reactivation.

Research Activities

Development of biomarkers based on DNA methylation in the NCAPH2/LMF2 *gene promoter region for diagnosing AD and amnesic mild cognitive impairment*

For the early treatment of dementia, a convenient method of diagnosis with biomarkers is required for AD and amnesic mild cognitive impairment (aMCI). To examine differences in DNA methylation due to AD and aMCI, we performed genome-wide screening by measuring blood DNA methylation levels with the Infinium HD Methylation Assay (Illumina, Inc., San Diego, CA, USA) in 3 groups of 4 subjects matched for age and sex: healthy control subjects and patients with aMCI or AD. The genome-wide analysis produced 11 DNA methylation loci that distinguished the 3 groups. For confirmation, we increased group sizes and examined samples by pyrosequencing, which revealed that DNA methylation in the non-SMC condensin II complex subunit H2 gene (*NCAPH2*)/lipase maturation factor 2 gene (*LMF2*) promoter region was significantly lower in patients with AD (n = 30) or aMCI (n = 28) than in healthy control subjects (n = 30) ($P < 0.0001$, analysis of covariance). No association was found between methylation levels and the apolipoprotein E genotype. We believe that the *NCAPH2*/*LMF2* methylation level might be a convenient and useful biomarker for diagnosing AD and aMCI.

Genetic association between presenilin 2 polymorphisms and AD and dementia of Lewy body type in a Japanese population

Background/Aims: Mutations of the presenilin 2 gene (*PSEN2*) cause familial AD. Common polymorphisms affect gene activity and increase the risk of AD. Nonsynonymous polymorphisms in the *PSEN2* have clinically shown Lewy body dementia (LBD) phenotypes. Therefore, we investigated whether *PSEN2* polymorphisms are associated with AD or LBD.

Methods: Seven single nucleotide polymorphisms (SNPs) of *PSEN2* were analyzed with a case-control study of 288 patients with AD, 76 patients with LBD, and 105 age-matched control subjects.
Results: Strong linkage disequilibrium was found from rs1295645 to rs8383 of *PSEN2*. The SNPs and AD onset were not associated, and genetic associations between AD and *PSEN2* were not detected. Although the number of cases was small, the SNPs studied did not modify the risk of LBD developing.
Conclusion: The common SNPs of *PSEN2* did not affect the risk of AD or LBD in a Japanese population. Because genetic variability of *PSEN2* is associated with behavioral and psychological symptoms of dementia in AD and LBD, further detailed analyses of the behavioral and psychological symptoms of dementia of both diseases should be performed.

Molecular mechanism of depressive disorder caused by HHV-6 latent infection
Background: Although stress is a major risk factor for depressive disorder, how stress induces depression is poorly understood. In our previous study, we showed that salivary HHV-6, which may invade the brain via the olfactory pathway, is increased by stress. Furthermore, we have identified small protein encoded by the intermediate stage transcript of HHV-6 (SITH) 1, which is produced specifically in the astrocytes during HHV-6 latency, and have found that patients with depression have antibodies to SITH-1.
Objective: To examine whether HHV-6 SITH-1 production in the olfactory system, which may be enhanced by stress, causes depressive disorder and to reveal the molecular mechanism by which SITH-1 induces depression.
Methods: A recombinant adenovirus carrying glial fibrillary acidic protein promoter-driven SITH-1 (SITH-1/Adv) was inoculated intranasally into C57BL/6 mice. A recombinant adenovirus without SITH-1 (control/Adv) was used in the control experiment. One week later, the tail suspension test was performed to assess the depressive-like behavior. Twenty-four hours later the olfactory bulb and brain were harvested for gene expression analysis. Depression-related messenger (m) RNAs were quantitated with the real-time reverse transcriptase-polymerase chain reaction.
Results and Discussion: In SITH-1/Adv mice, SITH-1 was detected with immunofluorescent staining in the olfactory epithelium. In the tail suspension test, immobility time was significantly greater in SITH-1/Adv mice than in control/Adv mice. The increase in immobility time was suppressed by pretreatment with an antidepressant agent (fluoxetine).Inoculation with SITH-1/Adv significantly increased expression of corticotropin-releasing hormone mRNA and, interestingly, significantly decreased bcl-2 mRNA and increased apoptotic cells (as indicated by terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate-fluorescein nick-end labeling) in the olfactory bulb. Overall, stress induces HHV-6 SITH-1 production in the olfactory system and subsequently induces brain cell apoptosis and corticotropin-releasing hormone overexpression, which may ultimately cause depressive disorder.

Publications

Suzuki A[1], Shibata N[1], Kasanuki K[1], Nagata T, Shinagawa S, Kobayashi N, Ohnuma T[1], Takeshita Y[1], Kawai E[1], Takayama T[1], Nishioka K[1], Motoi Y[1], Hattori N[1], Nakayama K, Yamada H, Arai H[1] ([1]Juntendo Univ). Genetic association between presenilin 2 polymorphisms and Alzheimer's disease and dementia of Lewy body type in a Japanese population. *Dement Geriatr Cogn Dis Extra.* 2016; **6:** 90–7.

Kobayashi N, Shinagawa S, Nagata T, Shimada K, Shibata N[1], Ohnuma T[1], Kasanuki K[1], Arai H[1], Yamada H, Nakayama K, Kondo K ([1]Juntendo Univ). Development of biomarkers based on DNA methylation in the NCAPH2/LMF2 promoter region for diagnosis of Alzheimer's disease and amnesic mild cognitive impairment. *PLoS One.* 2016; **11:** e0146449.