# Department of Virology

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## 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.

## Research Activities

*Reduction of adverse effects by a mushroom product, active hexose-correlated compound, in patients with advanced cancer during chemotherapy: The significance of the levels of HHV-6 DNA in saliva as a surrogate biomarker during chemotherapy*

Chemotherapy improves the outcome of cancer treatment, but patients are sometimes forced to discontinue chemotherapy or drop out of a clinical trial because of adverse effects, such as gastrointestinal disturbances and suppression of bone marrow function. The objective of this study was to evaluate the safety and effectiveness of a mushroom product, active hexose correlated compound (AHCC), on chemotherapy-induced adverse effects and quality of life (QOL) in patients with cancer. Twenty-four patients with cancer received the first cycle of chemotherapy without AHCC and then received the second cycle with AHCC. During chemotherapy, we evaluated adverse effects and QOL weekly via a blood test, European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) questionnaire, and DNA levels of HHV-6 in saliva. The DNA levels of HHV-6 were significantly increased after chemotherapy. Interestingly, administration of AHCC significantly decreased the level of HHV-6 in saliva during chemotherapy and improved hematotoxicity and hepatotoxicity, as well as QOL scores in the EORTC QLQ-C30 questionnaire. These findings suggest that salivary HHV-6 levels are a good biomarker of QOL in patients during chemotherapy and that AHCC has a beneficial effect on chemotherapy-associated adverse effects and QOL in patients with cancer undergoing chemotherapy.

*Effects of nutritional supplementation on fatigue, and autonomic and immune dysfunction in patients with end-stage renal disease: A randomized, double-blind, placebo-controlled, multicenter trial*

Background: Fatigue is a predictor of cardiovascular events in patients with end-stage renal disease (ESRD) undergoing hemodialysis. We hypothesized that multinutritional support would improve QOL, symptoms of fatigue, and potential quantitative measures, including endocrine, immune and autonomic functions, in patients with ESRD undergo-

ing hemodialysis.
Methods: A total of 202 patients undergoing hemodialysis were randomly assigned to receive active treatment (containing vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12, folic acid, vitamin C, carnitine, coenzyme Q10, naïve galacto-oligosaccharide, and zinc) or a placebo after each dialysis session for 12 weeks. The patients and attending physicians were blinded to the treatment, and 172 patients (86 in each group) completed the study. Fatigue was evaluated via fatigue questionnaire at 0, 4, and 12 weeks. To assess the reactivation of HHV-6 and HHV-7, the number of viral DNA copies in saliva was determined with the polymerase chain reaction at weeks 0 and 12. Autonomic function was determined via measurement of beat-to-beat variation with acceleration plethysmography.
Results: The groups did not differ significantly in clinical characteristics, changes in fatigue, QOL score, endocrine functions, or laboratory data. Several measures of heart rate variability significantly increased after nutritional treatment compared with those in patients receiving placebo. Consumption of a nutritional drink for 12 weeks significantly suppressed HHV-7 DNA copy numbers. Similarly, HHV-6 DNA copy numbers tended to be decreased by treatment but without reaching statistical significance.
Conclusions: Nutritional supplementation may modulate immune and autonomic dysfunction in patients with ESRD undergoing hemodialysis.

*HCMV latency-associated protein open reading frame 152 induces calcium influx and inhibits gene expression in nerve cells*

The virus known as HCMV is the most common cause of congenital virus infection. Congenital HCMV infection causes birth defects and development abnormalities, including sensorineural hearing loss, microcephaly, intracranial calcification, and intrauterine growth retardation. However, the pathogenic mechanism remains unclear. We have previously found that the HCMV latency-associated protein open reading frame (ORF) 152 can interact with calcium modulating cyclophilin ligand (CAML), a cellular protein that regulates the intracellular calcium concentration, and significantly enhance intracellular calcium concentration in granulocyte-macrophage progenitor cells, which are sites of latent HCMV infection. Because calcium has tremendous effects on neural activities, we investigated the effect of ORF152 on neural abnormalities.
First, we examined whether HCMV latency-associated protein ORF152 is expressed in nerve cell lines. Next, we introduced ORF152 stably into the U373, A172, and SVGp12 cell lines and measured intracellular calcium concentration. Previously CAML has been reported to be required for efficient recycling of epidermal growth factor receptor (EGFR). Therefore, we determined the expression level of EGFR in these stable cell lines.
We found that ORF152 could be expressed around the nucleus in HCMV-infected nerve cell lines. Moreover, ORF152 induced intracellular calcium concentration and, when stably expressed, inhibited EGFR expression in nerve cell lines.
These results demonstrate that the HCMV latency-associated protein ORF152 induces intracellular calcium concentration and inhibits EGFR expression in nerve cell lines. It has been reported that EGFR knock-out mice have defects in cortical neurogenesis.

Therefore, ORF152 is suggested to have effects on nervous system and is a key factor of neuropathogenesis during congenital HCMV infection.

*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

***Nagata T, Kobayashi N, Ishii J, Shinagawa S, Nakayama R, Shibata N[1], Kuerban B[1], Ohnuma T[1], Kondo K, Arai H[1], Yamada H, Nakayama K ([1]Juntendo Univ).*** Association between DNA methylation of the BDNF promoter region and clinical presentation in Alzheimer's disease. *Dement Geriatr Cogn Dis Extra*. 2015; **5:** 64-73.

***Fukuda S, Koyama H, Kondo K, Fujii H, Hirayama Y, Tabata T, Okamura M, Yamakawa T, Okada S, Hirata S, Kiyama H, Kajimoto O, Watanabe Y, Inaba M, Nishizawa Y.*** Effects of nutritional supplementation on fatigue, and autonomic and immune dysfunction in patients with end-stage renal disease: a randomized, double-blind, placebo-controlled, multicenter trial. *PLoS One*. 2015; **10:** e0119578.

***Ito T, Urushima H, Sakaue M, Yukawa S, Honda H, Hirai K, Igura T, Hayashi N, Maeda K, Kitagawa T, Kondo K.*** Reduction of adverse effects by a mushroom product, active hexose correlated compound (AHCC) in patients with advanced cancer during chemotherapy — the significance of the levels of HHV-6 DNA in saliva as a surrogate biomarker during chemotherapy. *Nutr Cancer*. 2014; **66:** 377-82.