Department of Virology

Kazuhiro Kondo, *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 the pathogenesis of HHV-6 and searching for latent proteins 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

Novel HHV-6 stress-related latent protein induces depression and suicide

Background: HHV-6 is one of a few viruses that establish latency within the brain. Latent infection occurs in astrocytes, and chronic stress can cause virus reactivation. However, the effects of reactivation in individuals with normal immune function were previously unknown. We identified the HHV-6 latent transcript that was expressed during the relatively activated latent stage (intermediate stage) of HHV-6 latency. This transcript encoded a small open reading frame, which we named small protein encoded by the intermediate-stage transcript of HHV-6 (SITH) 1.

Objective: To clarify the function of SITH-1 in the brain.

Methods: We studied the expression of the SITH-1 by examining the prevalence of antibodies against it among suicide attempters and healthy individuals. Next, an open reading frame of SITH-1 was linked downstream of a glial fibrillary acidic protein promoter and expressed in glial cells of mice using an adenovirus vector. After growth, the behavior and gene expression of the mice were analyzed.

Results and Discussion: The positivity rate of the anti-SITH-1 antibody was high in suicide attempters, especially those in whom depression had been diagnosed. Mice expressing SITH-1 showed a decrease in spontaneous motor activity and an increase in immobility time in the tail suspension test. Moreover, SITH-1 mice showed reduced expression of brain-derived neurotrophic factor messenger (m) RNA. Therefore, astrocytes expressing SITH-1 seem to play a major role in depressive-like behavior in mice. These results suggest that SITH-1 is involved in the onset of mood disorders. Our findings suggest that SITH-1 expression itself induces depression and, therefore, is a risk factor for depression and suicide.

Molecular mechanism of depressive disorder caused by latent infection with HHV-6

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 SITH-1, which is produced specifically in astro-

cytes 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 mRNAs 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 increases 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.

Human cytomegalovirus latency-associated protein ORF152 induces calcium signaling and promotes differentiation of myeloid progenitor cells

Human cytomegalovirus (HCMV) infection of healthy individuals is usually asymptomatic and results in the establishment of a life-long latent infection. Granulocyte-macrophage progenitors are sites of latent HCMV infection. The viral genome persists in these cells with highly restricted viral gene expression and no detectable virus production. Viral reactivation from latency is closely associated with cell differentiation. Sense and antisense CMV latency-associated transcripts, which are detected in latently infected cells, have been mapped to the ie1/ie2 region of the HCMV genome. One of the antisense transcripts, ORF152, is conserved among HCMV strains, but its role during latency remains unclear.
Here, we report the function of the HCMV ORF152 protein, which is expressed during latent infection. A yeast 2-hybrid screen showed that ORF152 bound to calcium-modulating cyclophilin ligand (CAML), a cellular protein that regulates the intracellular Ca^{2+} concentration.
Like that of CAML, expression of ORF152 significantly enhanced the kinetics and amplitudes of the increase in intracellular Ca^{2+} concentration upon stimulation with thapsigargin, a specific and irreversible inhibitor of endoplasmic reticulum Ca^{2+}-APTases. This finding indicates that ORF152 targets cellular CAML to increase the cytosolic Ca^{2+} response.

We also showed that ORF152 acted synergistically with CAML to activate the promoters activator protein 1 (AP-1) and nuclear factor of activated T cells (NFAT), which are related to the calcium-signaling pathway.

To address how ORF152 affects cell differentiation during latent HCMV infection, we induced ORF152 stably into HL-60 cells, which serve as a model for granulocyte-macrophage progenitors. With the addition of ORF152, HL-60 cells showed characteristics of mature macrophage/dendritic cells, such as enhanced expression of cell-surface markers CD80, CD83, and CD86. Maturation of granulocyte-macrophage progenitors is thought to be essential for HCMV reactivation.

Taken together, these results demonstrate that the HCMV latency-associated protein ORF152 induces intracellular calcium concentration, promotes cell differentiation, and acts as key trigger of reactivation from latency.

Publications

Nagata T, Kobayashi N, Shinagawa S, Yamada H, Kondo K, Nakayama K. Plasma BDNF levels are correlated with aggressiveness in patients with amnestic mild cognitive impairment or Alzheimer disease. *J Neural Transm.* 2014; **121:** 433-41. Epub 2013 Nov 20.

Tamai M, Namba H, Otsubo C, Wada Y, Kubo M, Ida H, Kobayashi N, Kondo K. A case of acute encephalopathy with febrile convulsive status epilepticus caused by HHV-6 reactivation (in Japanese). *Nihon Shonika Gakkai Zasshi.* 2013; **117:** 1459-63.