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General Summary

Human herpesvirus (HHV-) 6 is capable of establishing a lifelong latent infection of their host, is reactivated frequently. We are studying the molecular mechanism of latency and pathogenesis of HHV‐6, and find a novel latent protein of HHV‐6 which associate with chronic fatigue syndrome (CFS) and mood disorders. We are also attempting to apply HHV‐6 and HHV‐7 as tools for studying the mechanism of fatigue and as viral vectors for gene therapy.

Fatigue is an indispensable biological "alarm" for avoiding the state of exhaustion that is caused by severe stress and overwork and might also induce a variety of diseases. We have investigated the molecular mechanisms of HHV-6 and HHV-7 reactivation, which is known to be stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue.

Using our understanding of herpesvirus reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amount of a HHV-6 and HHV-7, which are reactivated and released into the saliva.

Research Activities

Identification of small protein encoded by the intermediate transcript of HHV-6 1 as a novel latent protein of HHV-6 associated with CFS and mood disorders

HHV-6 has exhibited the most promise as a candidate for a CFS-associated virus. We identified the novel HHV-6 latent transcript that was expressed during the relatively activated latent stage (intermediate stage) of HHV-6 latency. This transcript encoded the small open reading frame named small protein encoded by the intermediate transcript of HHV-6 (SITH)-1. In the present study we aimed to determine how SITH-1 is involved in CFS. In addition, to determine the function of SITH-1 in the brain, we analyzed the behavior of mice whose brains expressed SITH-1.

We studied the expression of SITH-1 by examining the prevalence of anti-SITH-1 antibodies in patients with CFS or mood disorders and in healthy persons. Antibodies were detected by means of indirect immunofluorescence and enzyme-linked sorbensorbent assay (ELISA). Next, an open reading frame of SITH-1 was linked downstream of a glial fibrillary acidic protein promoter and was expressed in glial cells of mice with an adenovirus vector. As they matured, the mice were analyzed with the tail suspension test and for prepulse inhibition and locomotor activity.

With the indirect fluorescent antibody method, rates of positivity for anti-SITH-1 antibodies were high in patients with CFS or mood disorders. In addition, the results of ELISA were highly correlated with those of indirect immunofluorescence. In behavioral experiments, 3-week-old SITH-1 mice showed decreased immobility time on the tail suspension

test and showed impaired prepulse inhibition. Meanwhile, 5-week-old SITH-1 mice showed a decrease in spontaneous motor activity and an increase in immobility time on the tail suspension test. Therefore, astrocytes exposed to SITH-1 seem to play a major role in the depressive and manic-like behavior of mice. These results suggest that SITH-1 is involved in the onset of mood disorders.

Application of HHV-6 as a gene therapy vector

HHV-6 is a lymphotropic virus that causes mild disease and has thus attracted interest as a viral vector for gene therapy. We have generated a recombinant HHV-6 virus named H6R28. In the present study, we used the recombinant HHV-6 as a gene therapy vector. In H6R28, HHV-6 U2-U8 genes were replaced with therapeutic genes and selection markers. H6R28 and the wild-type virus showed similar levels of viral replication in the phytohemagglutinin-stimulated umbilical cord blood mononuclear cells. On the other hand, replication of H6R28 was significantly impaired in adult human T cells, which is considered advantageous for developing a viral vector.

We generated a replication-defective HHV-6 vector expressing short hairpin RNA driven by a U6 promoter directed against the cellular molecule CD4 and human immunodeficiency virus (HIV)-1 core protein p24 Gag. In adult human T cells infected with the H6R28 expressing short hairpin RNA, fluorescence-activated cell sorter (FACS) analysis showed greater than 90% infectivity in both CD4-positive T lymphocytes and CD8-positive T lymphocytes. In infected cells a 90% decrease in CD4 expression and an 83% decrease in HIV-1 p24 expression were observed.

Replication-defective H6R28 showed a moderate infection rate in mature dendritic cells (DCs), a finding that suggests H6R28 could be used for anticancer DC therapy. In infected DCs, FACS analysis showed the stable expression of HLA-ABC and HLA-DR, which are usually down-regulated in herpesvirus-infected cells. These findings suggest that antigen-presenting capacity was maintained in DCs infected with replication-defective HHV-6. Therefore, we believe that H6R28 shows great promise as a gene therapy vector for acquired immunodeficiency syndrome and cancer.

Molecular mechanism and major cause of fatigue

Fatigue is an indispensable biological alarm to avoid the exhaustive state caused by severe stresses and overwork and might also induce a variety of diseases. Different types of fatigue might share a common mechanism.

For years many scientists thought that lactic acid caused fatigue. However, it is now understood that lactic acid itself does not cause fatigue, because lactic acid is a key substance for providing energy and because the acidity produced by a build-up of lactic acid helps prevent muscle fatigue. Thus, the molecular mechanisms of fatigue remain unclear.

We have investigated the molecular mechanism of herpesvirus reactivation, which is stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue. The molecule was up-regulated more than 10-fold with fatigue induced by forced swimming or sleep deprivation.

Our study describes a novel signal transduction pathway for fatigue and its relationship

with possible fatigue-causing substances, such as cytokines, and oxidative stress.

Reviews and Books

Kondo K. Human HHV-6 and 7: latency, reactivation and diseases (in Japanese). *Kagaku Ryoho no Ryoiki* 2010; **26:** 1211–7.

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