Department of Virology

Kazuhiro Kondo, *Professor*

General Summary

Human herpesvirus (HHV) 6, which can establish lifelong latent infections of hosts, is frequently reactivated. We are studying the molecular mechanisms of the latency and pathogenesis of HHV-6. Additionally, we are attempting to apply HHV-6 and HHV-7 to study of the mechanism of fatigue and to their use as viral vectors for gene therapy. Fatigue is an indispensable biological alarm to avoid the exhaustive state caused by severe stress and overwork, which may also induce a variety of diseases. 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. Using our understanding of herpesvirus reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amount of HHV, which is reactivated and released into saliva.

Research Activities

Development of a method for measuring accumulation of fatigue

The accumulation of fatigue causes many serious problems, such as death from overwork, suicide, and lifestyle-related diseases. However, scientific and medical studies of fatigue are not as advanced as those of other subjects, and not even a partial solution has been obtained for problems caused by fatigue in the health of individuals and society as a whole. The main reason for the delay in studies of fatigue and the development of methods for preventing and treating it is that a method for objectively measuring fatigue has not been proposed. In particular, a method for measuring the accumulation of fatigue or medium- and long-term continuous fatigue, which most severely affects life and health, has not been established, despite the high demand for such a method.

Using our understanding of herpesvirus reactivation, we have developed a method for measuring the accumulation of fatigue by determining the amount of a human herpesvirus, which is reactivated and released into saliva. Our study has revealed that fatigue accumulates due to various types of stress for a period of 1 week or more can be quantitatively determined with this method.

Development of a safe and simple technique for increasing the concentration of a virus or viral vector

Various viral vectors have been developed for genetic therapies and vaccine therapies, and positive results from their use are expected. However, because desirable effects cannot be obtained when the concentration of a viral vector is low, the concentration must be increased to an adequate level. Because the success of genetic therapy depends on the efficiency of gene introduction into cells, viral vectors that can introduce genes in

a highly efficient manner are used. However, retroviral vectors, including lentiviral vectors, showing high titers have not been obtained; hence, the efficiency of gene introduction is not sufficient. Accordingly, various processes for concentrating viruses and products based on such processes have been developed, but various practical remain. We have developed a novel method for increasing the concentration of a virus or a viral vector while maintaining its infective ability and have developed a kit for it. This method is a safe and simple way to increase the concentration of a vector, such as a retroviral vector or a herpes viral vector, useful for genetic therapy, vaccines, and the like.

Molecular mechanism and major cause of fatigue

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

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 through 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 by more than 10 fold with fatigue induced by forced swimming or shortened sleep.

Our study describes a novel signal transduction pathway for fatigue and its relationship with possible fatigue-causing substances, such as cytokines, and oxidative stress.

Identification of novel HHV-6 latent protein associated with mood disorders and the molecular mechanism of fatigue due to overwork

Mood disorders, such as depression, are frequently associated with chronic fatigue syndrome (CFS) and the physiological fatigue due to overwork. Viral reactivation of HHV-6 is a possible cause of CFS, and latent HHV-6 is reactivated by overwork. We searched for peptides encoded by HHV-6 that might produce the symptoms of CFS and for the molecular mechanism of fatigue due to overwork.

We have searched for novel HHV-6 latency-associated transcripts (H6LTs) and proteins and analyzed the function and prevalence of the newly identified latency-associated protein. Then, we studied the gene regulation of H6LTs, and searched for the factor(s) that induces viral reactivation.

We identified a 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 of a peptide, which we named small protein encoded by the intermediate stage transcript of HHV-6 (SITH)-1. SITH-1 significantly up-regulated the intracellular calcium levels of astrocytes. Mice expressing SITH-1 showed manic behavior. Serological studies identified antibodies against SITH-1 in 71% of patients with CFS and depressive symptoms, 53% of patients with depression, 76% of patients with bipolar disorder, 2% of healthy adults.

HHV-6 reactivation during fatigue was controlled by a small upstream open reading frame regulation mechanism that was released by the kination of eukaryotic initiation factor 2 alpha, which is a stress response mechanism in primitive eukaryote yeast.
We have identified the novel HHV-6 latent protein SITH-1, which may cause mood disorders. Furthermore, we have identified the molecular mechanism of fatigue that induces HHV-6 reactivation.
In this study we have shown that SITH-1, a protein encoded by HHV-6 during the intermediate stage of latency, is associated with mood disorders in CFS, depression, and bipolar disorder. Moreover, newly identified molecular mechanisms of fatigue may be related to HHV-6 regulation and mood disorders.

Use of HHV-6 and HHV-7 as gene therapy vectors
Recent technological advances in molecular biology and molecular genetics have contributed greatly to the recent progress in life sciences and have provided abundant information about various biological phenomena. Research has continued in the life sciences, with particular interest in the analysis of gene functions. This research has led to the development of techniques and vectors for introducing isolated genes into cells and organisms.
Viral vectors have advantages over other known vectors in introducing foreign genes into cells for protein expression. The central idea underlying gene transfer with viral vectors is to introduce a foreign gene into an infected cell and to transform the cell with the foreign gene under the control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, abortive infection).
In particular, HHV-6 and HHV-7 have attracted interest as viral vectors for gene therapy, because infections by these viruses cause only mild symptoms. Using the herpesvirus, and HHV-6 and HHV-7 in particular, as recombinant viruses and a recombinant viral vectors has certain advantages, which include low pathogenicity, the ease of introducing genes into such blood cells as the T cells and macrophages, and the ability to introduce large genes. However, producing a recombinant virus is difficult, and at present, no method is available to produce such recombinant viral vectors from HHV-6 or HHV-7. One factor that makes the recombination of HHV-6 and HHV-7 difficult is the characteristics of the HHV-6 and HHV-7 genes.
We have identified the dispensable genes of HHV-6 and HHV-7 and have reported the establishment of recombinant HHV-6 and HHV-7. The dispensable locus of HHV-6 is approximately 8.4 kbp, and that of HHV-7 is approximately 7.3 kbp; both are useful sites for inserting large genes. The exogenous nucleotide sequence may encode at least one kind of substance selected from a group consisting of a bacterial artificial chromosomes, cytokine genes, ribozymes, interference RNA, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, the exogenous nucleotide sequence may be used for the gene therapy of mammals. The gene therapy may be for preventing human immunodeficiency virus (HIV) infection of a compromised cell caused by HIV or for the immunotherapy of cancer.

Publications

Kondo K. Biomarker for fatigue: Human herpesvirus 6 (HHV-6) reactivation in saliva (in Japanese). *Igaku no Ayumi* 2009; **228:** 664–8.