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General Summary

Human herpesvirus (HHV) 6 belongs to the human β-herpesvirus subfamily, which includes human cytomegalovirus, HHV-6, and HHV-7. The β-herpesviruses can establish a lifelong latent infection of their host and are reactivated frequently, and some evidence suggests that the molecular mechanisms of viral latency and reactivation are common among these viruses. We are studying the molecular mechanisms of latency and pathogenesis of β-herpesviruses. Additionally, we are trying to apply the viruses as tools for studying the mechanisms of fatigue and as vectors for gene therapy.
Fatigue is an important warning sign of the exhaustive state caused by severe stresses and overwork, which may also induce a variety of diseases. We have investigated the molecular mechanism of herpesvirus reactivation, which is known to be stimulated by fatigue, and identified the molecule that can induce viral reactivation during fatigue.

Research Activities

Molecular mechanisms and major causes of fatigue
Fatigue is an important warning sign of the exhaustive state caused by severe stresses and overwork, which may also induce a variety of diseases. Different types of fatigue might have mechanisms in common.
For many years, lactic acid was thought to cause fatigue. However, lactic acid is now known not to cause fatigue, because lactic acid is a key substance that provides energy and because acidity caused through a build-up of lactic acid helps prevent muscle fatigue. Thus, the molecular mechanisms of fatigue have become unclear.
We have investigated the molecular mechanism of herpesvirus reactivation, which is known to be 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 includes the novel signal transduction pathway of fatigue and its relationship with candidate fatigue-causing substances, such as cytokines, and oxidative stress.

Identification of novel HHV-6 latent protein associated with mood disorders and molecular mechanism of fatigue due to overwork
Mood disorders, such as depression, are frequently associated with chronic fatigue syndrome (CFS) and 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 newly identified latency-associated protein. We then studied the gene regulation of H6LTs and searched for the factor of factors that induce viral reactivation.
Results: 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 that we named "small protein encoded by the intermediate stage transcript of HHV-6 (SITH)-1." SITH-1 significantly upregulated the intracellular calcium levels of astrocytes. Mice expressing SITH-1 showed manic behavior. A serological study showed that antibodies against SITH-1 were present in 71% of patients with CFS who had depressive symptoms, 53% of patients with depression, and 76% of patients with bipolar disorder, and 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 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.
Discussion: 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, a newly identified molecular mechanism of fatigue may be related to HHV-6 regulation and mood disorders.

Latency and reactivation of β-herpesviruses
Both HHV-6 and HHV-7 are almost universally acquired by 2 to 3 years of age. These viruses belong to the β-herpesvirus subfamily and are closely related to each other, on the basis of biological and molecular analyses. They establish life-long latency, a hallmark of herpesviruses, reactivate frequently, and are shed in saliva.
To investigate viral reactivation, we have identified the latency-associated transcripts of HHV-6 and have partially clarified the mechanism of HHV-6 reactivation. HHV-6 established latency in macrophages and kept a fairly stable intermediate stage between latency and reactivation, and the viral reactivation was induced by 2 or more factors.
HHV-6 can reactivate in immunosuppressed patients; however, the relationship between immunosuppression and the induction of reactivation is unclear. To identify the factor(s) of HHV-6 reactivation, we have studied the association between HHV-6 reactivation and work-induced fatigue in healthy adults. Immune strength is thought to deteriorate when humans are fatigued, and viral infection reflects this deterioration of immune strength. However, a relationship between fatigue and viral infection in humans has not been proven.
The DNA of HHV-6 was detected in 88% of subjects engaged in moderately intense work due to prolonged working time and other factors (the first test day). In contrast, HHV-6 DNA was detected in 23.8% of subjects immediately after a holiday (the second test day). These results show that HHV-6 is significantly reactivated on exertion. These results led to the discovery that HHV-6 DNA expressed in saliva through the

reactivation of HHV-6 is a fatigue biomarker (a biological index factor) that varies with the degree of fatigue. Accordingly, an objective method for assessing the degree of fatigue by detecting HHV-6 DNA released into saliva as a result of the reactivation of HHV-6 was developed, enabling a simple and easy method of assessing the degree of fatigue.

The amount of HHV-7 DNA was semiquantitatively measured with a double-nested polymerase chain reaction method after serial dilution of saliva. HHV-7 DNA was detected in 92% of patients with CFS. In contrast, HHV-7 DNA was detected in 50% of healthy subjects during work and in only 30% of healthy subjects at rest. The amount of HHV-7 DNA in half of the patients with CFS were 10 to 100 times greater than the mean amount in healthy subjects. These results show that HHV-7 is significantly reactivated in the chronic fatigue state that accompanies disease. These results led to the discovery that HHV-7 DNA expressed in saliva due to reactivation of HHV-7 is a fatigue biomarker (a biological index) that varies according to chronic fatigue caused by diseases or other factors. Accordingly, an objective method for assessing the degree of fatigue by detecting HHV-7 DNA released into saliva due to reactivation of HHV-7 was developed, enabling a simple and easy method of assessing the decrease in physical strength caused by chronic fatigue.

Application of HHV-6 and HHV-7 as gene therapy vectors

Accumulation of knowledge and various technological advances in molecular biology and molecular genetics have greatly contributed to the recent progress in life science, providing abundant information about various biological phenomena. Active research and development has continued in various fields of life science, with particular interest in the analysis of gene functions. This has led to the development of techniques and vectors for introducing isolated genes into cells and individual organisms.

Virus vectors have advantages over other known vectors in introducing a foreign gene into a cell for protein expression. The central idea underlying gene transfer with a virus vector is to introduce a foreign gene into an infected cell and transform the cell with the foreign gene under control of promoter sequences, taking advantage of the infectious capacity of the virus (productive infection, latent infection, abortive infection).

HHV-6 and HHV-7, in particular, have attracted much attention as candidate virus vectors for gene therapy, because infections with these viruses cause mild symptoms. Using herpesviruses, and HHV-6 and HHV-7 in particular, as recombinant viruses and recombinant virus vectors has advantages, which include low pathogenicity, ease of gene introduction into blood cells, such as T cells and macrophages, and introduction of relatively large genes. However, it is difficult to produce recombinant viruses and recombinant virus vectors that originate in HHV-6 or HHV-7, and no method available today can produce such viruses and vectors. One factor that makes recombination of HHV-6 and HHV-7 difficult, in addition to technical factors, is the characteristics of 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 1 type of substance selected from a group including bacterial artificial chromosomes, cytokine genes, ribozymes, interference RNA, immunological co-stimulator molecules, signal transduction molecules, enzymes, and chemical attractants. Furthermore, exogenous nucleotide sequences may be used for the gene therapy of mammals. Gene therapy might be used to prevent human immunodeficiency virus infection of a compromised cell caused by human immunodeficiency virus and for the immunotherapy of cancer.

Publications

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