News Release

Title

Identification of the cause of chronic active Epstein–Barr virus (EBV) infection and the mechanism by which EBV causes cancer

Key Points

○ A research group in Japan, led by Dr. Hiroshi Kimura, has elucidated the cause of chronic active Epstein–Barr virus (EBV) infection.

○ The study reveals why EBV causes cancer in only a small subset of infected individuals.

○ The findings of the study is expected to lead to the development of therapeutics for chronic active EBV infection and EBV-associated cancers.

Summary

Dr. Yusuke Okuno at the Department of Advanced Medicine, Nagoya University Hospital (Director, Naoki Ishiguro, M.D., Ph.D.); Prof. Hiroshi Kimura at the Department of Virology, Nagoya University Graduate School of Medicine (Dean, Kenji Kadomatsu, M.D., Ph.D.); Prof. Takayuki Murata at the Department of Virology and Parasitology, Fujita Health University; Prof. Seishi Ogawa at the Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University; and Prof. Satoru Miyano at the Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, the University of Tokyo, along with their colleagues, conducted a comprehensive genetic analysis of chronic active Epstein–Barr virus infection (CAEBV) to elucidate its cause. Their study also clarified why EBV, which is not harmful to most people, causes cancer in a small subset of individuals. The results of this research was published in *Nature Microbiology* by *Nature Publishing Group* (January 21, 2019).

EBV infects 95% of humans and usually causes cold-like symptoms. However, in only a small number of individuals, EBV causes cancer and other intractable diseases. CAEBV is characterized by inflammation associated with EBV infection that lasts years and is related to various life-threatening complications. The cause of CAEBV is poorly understood.

The research group led by Dr. Kimura conducted a comprehensive genetic analysis using next-generation sequencing, which revealed that CAEBV is a type of hematological cancer. Their genetic analysis also revealed that the EBV strain associated with CAEBV lost several viral genes, i.e., becoming a defective EBV, and was abnormally activated; such activation was associated with cancer development. In

addition, the research group identified that this defective EBV was present in other hematological malignancies as well, suggesting that EBV could be causing various neoplasms using a common molecular mechanism.

Research Background

EBV infects 95% of humans, and usually occurs during childhood or adolescence. Most infections are self-limited; however, EBV causes cancer and other intractable diseases in only a limited number of individuals, and its cause is poorly understood.

CAEBV is a disease with unknown etiology, and several dozen cases of CAEBV are reported annually in Japan. The patients contract EBV infection that lasts for more than 10 years. The symptoms of CAEBV vary among patients; while some are asymptomatic, others experience continuous/repeated cold-like symptoms with or without symptoms specific to CAEBV including mosquito-bite allergy and/or hydroa vacciniform-like skin lesions. In CAEBV, EBV-infected cells invade and destroy multiple organs or abnormally proliferate and behave similar to a hematological malignancy. Several treatments such as chemotherapy and hematopoietic stem cell transplantation were reported to be effective; however, the prognosis remains poor despite appropriate treatment.

This research conducted a comprehensive genetic analysis of CAEBV, including the genomes of the patients and EBV, to elucidate the cause of this disease.

Research Results

This study included 80 patients with CAEBV using next-generation sequencing. Their analysis revealed that EBV-infected cells in patients with CAEBV acquired mutations which are usually observed in cancer. The mutations were most frequently observed in *DDX3X* gene, which is frequently mutated in other hematological cancers associated with EBV. It also became clear that patients harboring EBV-infected cells with *DDX3X* mutations at diagnosis had worse prognosis (Figure 1).

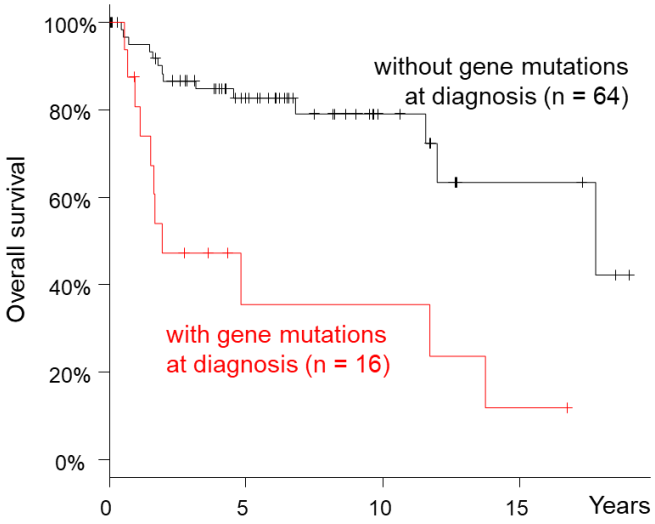

Figure 1. Relationship between gene mutations and prognosis at diagnosis
The horizontal axis shows the period since diagnosis, and the vertical axis shows the percentage of surviving patients. Patients carrying EBV-infected cells with gene mutations showed worse prognosis compared with others.

Next, the research group performed genetic analysis of a patient with CAEBV who progressed gradually to ultimately develop a hematological cancer (blastic crisis) (Figure 2); the EBV-infected cells in this patient acquired three different *DDX3X* mutations over time. In addition, the cells acquired a *PD-L1* mutation, which facilitates the escape of mutated cells from the immune system of the patient. These observations of the study group indicate that CAEBV behaves like cancer.

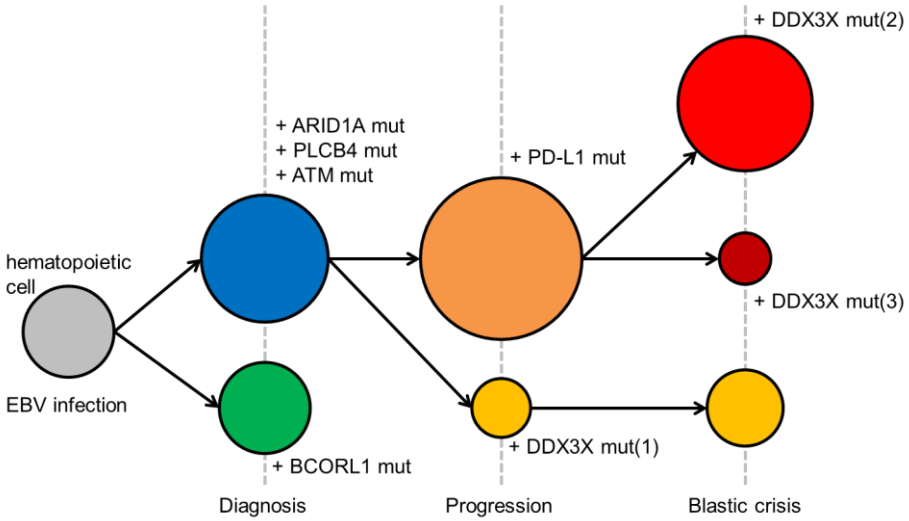

Figure 2. Acquired mutations in EBV-infected cells of a patient with CAEBV
As the disease progressed, EBV-infected blood cells acquired various genetic mutations. In particular, *DDX3X* gene acquired three mutations, which was considered to play an important role in the progression of CAEBV.

In addition to human genes, the research group analyzed all viral genes of EBV and identified that EBV strain in patients with CAEBV lost several viral genes; thereby becoming defective (Figure 3). The lost genes included those necessary for the latency of EBV in host cells and those required for the production of viral particles that infect other cells and transmission to other humans.

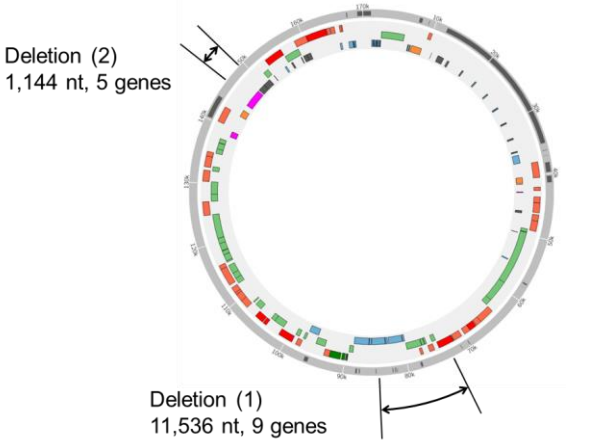

Figure 3. An example of a defective EBV found in a patient with CAEBV
The EBV genome is circular and contains around 80 genes. In this example, the two areas of the EBV genome are missing. There are nine genes in the first region, which contains essential genes to produce infectious viral particles. The second region contains genes necessary for viral latency in infected cells during latent infection.

Further experiments revealed that the defective EBV was activated abnormally to use virtually all viral genes, which promoted the evolution of infected cells to become cancerous. In addition, defective EBV was not only prevalent in CAEBV but in other EBV-associated hematological malignancies, such as EBV-positive diffuse large B-cell lymphoma and extranodal natural killer/T-cell lymphoma, suggesting that EBV utilizes the same mechanism to cause various hematological cancers.

Research Summary and Future Perspectives

The findings of this study illustrates that CAEBV exhibits several properties of cancer. In addition, the EBV strains involved in hematological cancers have lost several genes, leading to their abnormal activation to promote cancer. Further research is necessary to translate these findings to treatments. It may be possible to perform genetic analysis of patients with CAEBV at the time of diagnosis to predict prognosis or to determine if immune checkpoint inhibitors against *PD-L1* mutations can be utilized. These findings are also expected to translate to the development of therapeutic approaches to suppress the abnormal activation of defective EBV.

Publication

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