

Invited Paper

## Epstein-Barr Virus in Malignant Lymphoma

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### Abstract

Since its discovery in the cell line derived from an African Burkitt's lymphoma (BL) in 1964, the Epstein-Barr virus (EBV) is well known oncovirus associated with several human diseases. Malignant lymphoma is one of the most interesting diseases which is associated with EBV, because this virus primarily infects in lymphocytes.

Recent extensive pathology works on EBV-associated diseases are supported by the immunohistopathology using newly available antibodies for EBV-related proteins, polymerase chain reaction, and in situ hybridization, particularly targeted the EBV encoded small RNA (EBER). EBER in situ hybridization is very sensitive to detect EBV in routine formalin-fixed paraffin embedded pathology sections.

EBV is present in almost all of African BL, but is associated with low number of non-African BL. EBV is associated with lymphoproliferative disorders in the immunocompromized including post-transplant individuals and AIDS patients. Many of these EBV associated lymphomas are B-cell type.

EBV is also known to be associated with Hodgkin's disease, and some types of T-cell lymphoma including nasal T-cell lymphoma and sporadic other types of T-cell lymphoma with higher prevalence rate than B-cell lymphoma.

Oncogenicity of EBV in human diseases has been known by the evidences such as EBV genome of EBV encoded RNA present in all tumor cells by in situ hybridization, monoclonality of EBV-episomes by southern blot hybridization, expression of EBV-related oncogenes and oncoproteins, isolation of tumor formation in nonhuman primates, and EBV-infected

lymphocytes from healthy individuals can produce lymphomas in SCID mice<sup>1-3</sup>).

There have been dramatic increase in human diseases which is associated with EBV by the pathology works using recently available techniques such as polymerase chain reaction (PCR), in situ hybridization (ISH) and immunohistochemistry (IHC), to detect EBV-related proteins over the last few years<sup>4</sup>).

In addition to the Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC), gastric carcinoma and undifferentiated carcinoma with lymphoepithelioma-like histology of salivary gland, lung and thymus are known to be associated with EBV<sup>5</sup>).

Malignant lymphomas (ML) occurring in immunocompromized persons such as post-transplant individuals and AIDS patient, nasal T-ML, T-ML with angiocentric pattern, and T-ML including adult T-cell leukemia (ATL) have been reported to be linked with EBV<sup>6-9</sup>).

In this report, we summarize the recent works of EBV in ML with our own experiences.

**Key words:** Epstein-Barr virus, malignant lymphoma, EBER, in situ hybridization

### EBV biology and it's infection

EBV is a very large DNA virus with 172-kb sequences which is classified in herpesvirus type 4, and infects ubiquitously in human adults. EBV primarily infects by the transmission in saliva usually from parents to child with asymptomatic or minimal symptomatic. In western countries, EBV exposure is occur in adolescence or young adults, and these late infection commonly associated with infectious mononucleosis (IM)<sup>1</sup>).

EBV primary infection is thought to occur theoretically through two pathways of oropharyngeal epithelium and B-lymphocytes<sup>10</sup>). EBV infection of B-lymphocytes is mediated by the complement receptor

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CD21 (CR2). EBV infection in epithelial cells is thought to be mediated by the secretory IgA components which has been demonstrated *in vitro*<sup>11)</sup>.

EBV infected cells exhibit two different states of latent and lytic. In lytic state, infected cells produce of infectious virus. A small number of latently infected B-lymphocytes become immortalized with expression of several EBV proteins such as EB nuclear antigen-1 (EBNA-1), a specific DNA binding protein which is important in episomal replication, EBNA-2, a specific transcriptional transactivator of viral and cellular genes, and the latent membrane protein-1 (LMP-1), a transmembrane phosphoprotein that transform rodent cells<sup>11)</sup>. These EBV proteins (EBNA-2, LMP-1) are the major targets of cytotoxic T-cell response to EBV infected cells.

### EBER in situ hybridization

Although EBNA-1 is believed to be expressed in all forms of EBV latently infected cells and is the only well-characterized universal antigenic target for detection of EBV latent infection in tissue sections, there is no available antibodies to detect in formalin-fixed paraffin sections. This protein is expressed only low level which are able to stain by complement method on the frozen sections.

In situ hybridization of EBV-DNA has been applied in sometime to detect EBV infected cells, but the sensitivity is very low because the genome copy numbers in latently infected cells are usually low. There are two short viral transcripts that are abundantly expressed by EBV in latent infected cells, known as EB encoded small RNA (EBER). EBERs are transcribed by RNA polymerase III at similar rates. The EBER-1 level can be as high as  $10^7$  molecules per cell in some EBV-infected cell lines. The molecules of these transcripts are 167 and 172 nucleotide. These two RNA are also present in the cells with active EBV replication except of oral hairy leukoplakia cells of AIDS patients. EBERs are present even in BL which express only EBNA-1 gene.

Although the accurate function of these two transcripts have not been discovered, these RNAs may play an important role in maintaining the immortalized phenotype of EBV-infected cells since viruses generally do not express unnecessary genetic information<sup>12)</sup>. These two RNAs are the highly sensitive targets for in situ hybridization to detect EBV latently infected cells in routinely processed paraffin sections with preserving the morphology and phenotypic expression. Full-length antisense riboprobes or short oligonucleotide probes have been used. Probes could be labeled with isotope, digoxigenin, biotin, or fluorescein. The ISH signals of EBER-ISH are observed in the nuclei, particularly just inner side of the nuclear membrane and around the nucleolus with completely absent in the cytoplasm. These EBER-ISH shows no background signals in

unexpected tissue except a few crystal deposit and brown staining on the collagen tissue when used digoxigenin labelling<sup>5)</sup>. There are still possibility that EBER-ISH are not completely detect the EBV-infected cells because the intensity of the ISH signals vary from black to faint grey, suggesting the copy number of EBERs are variable by cell to cell even in the same tissue specimens. Then it is recommended to compare the EBER-ISH results with PCR in situ hybridization of EBV-DNA, particularly in the study of ML, because many T-ML express EBER-ISH signals sporadically in the tumor cells.

### EBV-infected cells in infectious mononucleosis

We have reported the results of dual staining of EBER-ISH and immunohistochemistry to detect the marker expression of EBER positive lymphocytes in IM<sup>13)</sup>. There are many EBV-positive T-lymphocytes as well as EBV-positive B-lymphocytes. Recently we also found EBV-positive macrophage in lymph node of IM (in preparation). Thus, even in the lesion of initial infection of EBV, T-cells and macrophage as well as B-cells may play an important role in the histogenesis of IM lymphadenitis and tonsillitis which have T-cell lymphoproliferative features on the histology.

T-cell lymphoproliferative disorders are also known in chronic active EBV infection<sup>14)</sup>. In this disorder, EBV infected T-cells are observed in the vascular wall with severe vasculitis which is similar to Kawasaki disease, in lungs and liver.

These findings of EBV-infected T-lymphocytes may suggest the possibility to play a causal role in the development of EBV positive T-cell lymphoma<sup>15)</sup>.

### EBV-associated lymphomas

#### Burkitt's lymphoma

The association of EBV with ML are known from the first discovery in the African BL. In BL, EBV are observed in the tumor cells uniformly by the EBER ISH. These cells also express EBNA-1 in the frozen section. There are cases with lytic state in BL cases. BL is thought to evolve multistep process involving malaria, EBV, genetic predisposition which has been suggested by HLA studies, other environmental agents and myc gene activation resulting from chromosomal translocation. BL cells only express EBNA-1 which is not recognized by cytotoxic T-cells, thus the EBV-infected tumor cells can escape from EBV-specific immune surveillance.

#### B-cell lymphoma in immunodeficiency individuals

The association of EBV in ML of post-transplant recipients have been known<sup>7)</sup>. These are large cell lymphomas with B-cell phenotype which are similar to the EBV-positive lymphomas occurring in AIDS patients<sup>6)</sup>. These lymphoma cells express EBNA-1,

EBNA-2, and LMP-1. AIDS associated lymphomas predominantly locate in central nervous system. These large cells lymphomas uniformly express EBERs and can easily demonstrate EBV by EBER-ISH. Because there is no other such lesions with uniform expression of EBER. EBER-ISH is one of the useful diagnostic technique to detect lymphoma in AIDS patients even in the small biopsy materials. The pathogenesis of EBV-associated lymphomas in immune compromised patient can be explained by the escape from cytotoxic T-cell mediated viral immune surveillance by the immunodeficiency although these tumor cells show several viral gene expression.

Post-transplantation patients usually develop EBV-infected benign lymphoproliferative disorder and rarely manifest their EBV-infection as a monoclonal B-cell proliferation and is termed as ML. Similar developing pattern of EBV-associated clonality pattern is also observed in AIDS patients.

#### **Pyothorax associated pleural B-cell lymphoma**

Demonstration of EBV in B-cell lymphoma occurring in pleura in patients having past history of pyothorax due to tuberculosis with a latent period of more than 40 years is also an interesting findings<sup>16-17</sup>. These patients do not show immunodeficiency, but the EBV expressing pattern is similar to the B-cell lymphomas arising in immunocompromized individuals. These EBV-positive lymphoma cells express EBNA-2, LMP-1, and EBER with high titer of EBV serology.

#### **Peripheral T-cell lymphoma**

The association of EBV with peripheral T cell lymphomas are known in cases of angiocentric lymphoma and nasal T cell lymphoma with or without NK marker expression<sup>18-20</sup>. This type of lymphoma was first reported in association with EBV for nasal lymphoma<sup>18</sup>, then lymphomas with angiocentric histology or CD56 positive lymphoma<sup>19-20</sup>. Similar lymphoma with angiocentric pattern of the lung is called as pulmonary lymphomatoid granulomatosis, which also has EBV-positive cells in B cells with a prominent T-cell components<sup>21</sup>. These lymphomas are characterized by the predominantly location in extranodal site, expression of T-cell markers except CD3, expression of CD56 in the most cases, with angiocentric pattern showing necrosis, and pleomorphic histology. These lymphomas are very aggressive and may be endemic in Asia. EBER ISH reveals diffuse uniform positive pattern in almost all lymphoma cells (Figure-1). Further study is recommended to elucidate the definition of these type of lymphoma and the role of EBV.

High frequency of EBV-positive lymphomas has been reported for peripheral T-cell lymphomas from China<sup>22</sup>. In our study, EBV is also observed in the specimen of adult T-cell leukemia/lymphoma with

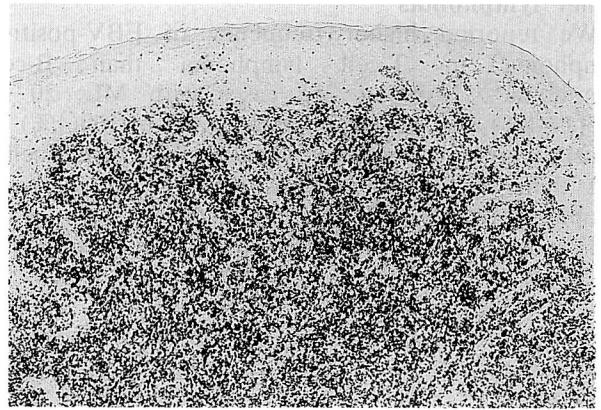


Fig. 1.

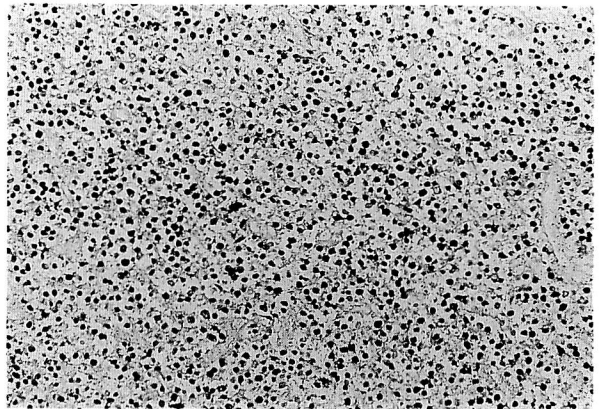


Fig. 2.

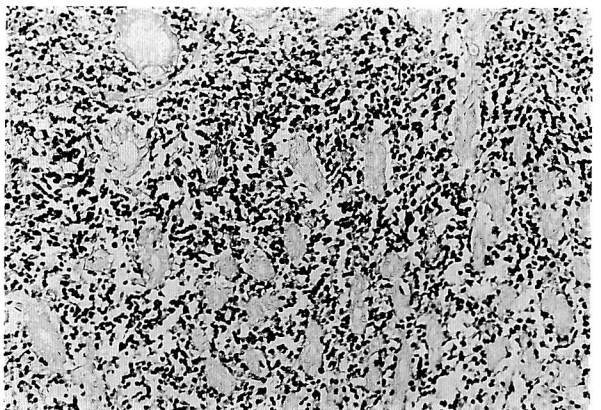


Fig. 3.

pleomorphic type (Figure-2)<sup>9</sup>). In these cases, EBER ISH positive signals are observed in the pleomorphic tumor cells. Some of these EBV-positive lymphoma cells express EBNA-2 and LMP-1, suggesting both HTLV-1 and EBV may play some role in the development of ATL development. There are some question for the understanding of the phenomenon because not all tumor cells express EBER.

### Other lymphomas

We reported higher frequency of EBV-positive lymphoma in T-cell lymphoma than B-cell lymphoma<sup>23)</sup>. Among 280 cases with ML, 20 of 175(11.4%) of T-cell lymphoma, and 4 of 100(4%) of B-cell lymphoma are EBV positive in the tumor cells, but there are some cases with EBV-positive non-neoplastic lymphocytes (bystander cells). There is no EBV-positive cases in follicle center cell lymphomas. There are a few cases with EBV-positive signals in MALT type lymphoma (Figure-3).

Our recent study indicates that the EBV-associated lymphoma cases show poor prognosis than that of EBV-negative cases, with episomal clonality, P-53 overexpression, and oncoprotein expression.

Further studies of clonality analysis, antibodies, gene expression, gene products expression, and oncogene are demanded for the understanding of EBV-associated lymphomas.

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