EPSTEIN-BARR VIRUS – MOLECULAR BASIS FOR MALIGNANT TRANSFORMATION

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ABSTRACT

Epstein-Barr (EBV) is a widespread virus which can be detected in more than 90% of world population. Primary EBV infection during adolescence and adulthood results in infectious mononucleosis, while in children it is usually asymptomatic. EBV is responsible for different malignant forms of B-cell or epithelial cancers, such as Hodgkin’s and non-Hodgkin’s lymphoma, Burkitt’s lymphoma, post-transplant lymphoproliferative disorders, nasopharyngeal carcinoma, hairy leukoplakia and HIV-associated lymphomas. Evidence exists that an infection with EBV is also linked with a higher risk of hepatocellular and gastric cancers, as well as autoimmune diseases.

EBV shows two alternative life cycles – latent and lytic. After the primary infection, the virus remains in the B lymphocytes in latency, while the lytic infection takes place predominantly in the epithelial cells and can last for months with constant virus release in saliva and nasopharyngeal secretion. Unlike other herpes viruses, the development of oncological diseases is linked with the latent cycle, as a result of the immune response failure to control latently infected cells.

With the present work we try to concisely review the current knowledge about mechanisms of EBV pathogenesis in humans and to summarize recent findings in the field.

Keywords: Epstein-Barr virus, EBV pathogenicity, EBV latency, malignant transformation

INTRODUCTION

EBV is a virus frequently found among the human population and transmitted mainly through saliva, blood transfusion or organ transplantation. Sexual transmission is also possible.

It is a complex, spherical virus with a size between 120 and 180nm. Principal structural elements of EBV include: (1) double-strand DNA nucleoid; (2) icosahedral capsid formed from 162 capsomeres; (3) lipoprotein envelope containing viral glycoproteins; and (4) tegument, placed between the capsid and the envelope (1,2). The genome of EBV is a linear double-strand DNA of 172 kb (for B-95-8 strain) or 184 kb (for other strains) (3) coding for 86 (4) or 100 viral proteins, respectively (3). These genes can be specific for Herpes viruses family, for Gammaherpesvirinae subfamily or for genus Lymphocryptovirus (5).
adulthood it is present as infectious mononucleosis (6). EBV is the first known human tumor virus (7) with high transforming activity towards lymphoid cells. The virus has two “in vivo” target tissues – B-lymphocytes and epithelial cells (8) with different corresponding mechanisms for adhesion and entry. The main receptor on B cells is CD21 (CR2 or complement receptor type 2) which interacts with the viral gp350/220 molecules, while still unknown receptor on epithelial cells is recognized by the gH/gL viral protein (9). Another possible mechanism for epithelial cell infection is the detection of EBV BMRF2 protein from betha-1 or alpha-5, betha-1 cellular integrins via IgA-mediated interaction (10-13). Some authors accept more efficient epithelial infection after preliminary adhesion of EBV to resting B-cells and consecutive transfer to the tolerant epithelium (14).

EBV shows two alternative life cycles – lytic and latent. The lytic infection takes place predominantly in the epithelial cells and can last for weeks or months with constant release of infectious viral particles in saliva and nasopharyngeal secretions (15,16). The primary infection of naive B-cells in the oropharynx is the most effective and leads to their constant proliferation (17,18), as the virus remains in the B-lymphocytes (latent infection) and its genome is located in the nucleus under the form of a circular episome. In immunocompetent individuals cytotoxic T cells (CTLs) and NK cells control the spread of EBV-infected B-lymphocytes and the virus is limited to the memory B-cells where it develops a latency program (19). Latently infected B-cells express only 10% of the EBV genome and rarely support the lytic life cycle of the virus (6,20).

EBV persists in memory B-cells in latency program 0 suppressing the expression of all latent genes except for Epstein–Barr virus-encoded small RNAs (EBERs) and BamHI A rightward transcripts (BARTs) (17,21). The lack of viral genes expression helps the virus to escape the immune response of the host. Under Latency 0 program, EBNA1 (EBV protein found in EBV-related tumorigenesis) is only expressed during cell division. Latency program I involves a minimal level of gene expression needed for EBV genome persistence in the host cell (22). The different latency programs of EBV, together with the latent gene expression and the corresponding diseases, are presented in Table 1.

<table>
<thead>
<tr>
<th>Latency program</th>
<th>EBNA-1</th>
<th>EBNA-2</th>
<th>EBNA-3</th>
<th>LMP-1</th>
<th>LMP-2</th>
<th>EBER</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Burkitt’s lymphoma</td>
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<tr>
<td>II</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Hodgkin’s lymphoma, nasopharyngeal carcinoma, peripheral T-cell lymphomas</td>
</tr>
<tr>
<td>III</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Lymphoproliferative disorders, X-linked lymphoproliferative disorders, HIV-associated lymphomas</td>
</tr>
<tr>
<td>other</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Healthy carriers</td>
</tr>
</tbody>
</table>

EBNA – EBV nuclear antigen, LMP – latent membrane protein, EBER – EBV-encoded small RNAs

Table 1. Different latency programs of EBV and gene expression in EBV-associated diseases (according to (6))

During the primary infection the infected B cells tend to spread rapidly and to infect other cells. The new infection is overcome by the cytotoxic T-lymphocytes and in immunocompetent individuals with lytic infection are rarely found (23). Despite the type of infection, EBV can persist in the cells to the end of the host’s life. Reactivation is a way to infect new lymphocytes and spread virus particles. It occurs during the recirculation of EBV-infected B-cells in the lymphoid tissue. B-cells react to different physiological stimuli – lack of CD40 activation, B-
cell receptor stimulation and LMP1 expression (24-26). As a result, a viral replication of ZEBRA (which activates the switch from the latent to the lytic gene expression program) and VCA (viral capsid antigen) starts to release new virions (24).

Unlike other herpes viruses, the development of EBV-related oncogenic diseases is associated with the latent life cycle of the virus. EBV-related malignant diseases occur when the immune response fails to control the spread of latently infected cells. At least 5 genes are involved in the process of malignization by blocking tumor-suppression cell mechanisms (as is the case of other DNA tumor viruses). In general, EBV is less cancerogenic than the small oncogenic DNA viruses (27). Malignant transformation of EBV is in combination with other factors, such as strong immune deficiency (28,29). The time between the primary infection and the malignization depends on geographic and genetic factors (30-32).

During the primary infection, several EBV genes are expressed – some of the most important ones are EBNA-1 and LMP-1 (27,33). LMP-1 is a primary oncogene – transgenic mice expressing the corresponding protein soon develop lymphomas. The product of the gene is detected in most of the EBV-associated oncogenic diseases (Hodgkin’s lymphoma, post-transplant lymphoproliferative disorders, nasopharyngeal carcinoma and HIV-associated lymphomas). Its expression causes changes related to B-cell activation, including B-cell fusion, augmentation of CD23, CD39, CD40, CD44 expression and anti-apoptotic effects (via increase in the BCL-2 and A20 levels) (34,35). LMP1 functions as constitutively active receptor in the family of tumor necrosis factor receptors (TNFR) and activates cell signaling pathways via ligand-independent way by mimicking the active CD40 (36,37). Its activity is associated with at least four cell-signaling pathways – NF-kB, JNK (AP-1, p38 ), MAPK and JAK/STAT (38,39). LMP1 stimulates cytokines production (IL-6, IL-8) via interaction with p38 mitogen-activated protein kinase pathway or NF-kB signaling pathway in epithelial cells (40). Phosphatidylinositol 3-kinase (PI3-K) pathway could also be activated, leading to cell survival. The activity of LMP1 and other essential latent viral proteins is listed in Table 2.

### Table 2. Essential latent proteins of EBV (according to (3)).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Localization</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>EBNA-1</td>
<td>Nuclear</td>
<td>Latent phase viral DNA replication: binds the viral latent origin, oriP; activates transcription of other latent genes</td>
</tr>
<tr>
<td>EBNA-2</td>
<td>Nuclear</td>
<td>Transcriptional activator of viral and cellular genes: targeted at DNA by the cellular J kappa recombination signal sequence binding protein (RBPJ)</td>
</tr>
<tr>
<td>LMP-1</td>
<td>Plasma membrane</td>
<td>Transcriptional activator: engages signaling proteins for the tumor necrosis factor receptor family; cause B cell activation and differentiation; prevents apoptosis</td>
</tr>
<tr>
<td>EBNA-3C</td>
<td>Nuclear</td>
<td>Transcriptional activator of CD21, LMP1, fine control of EBNA2</td>
</tr>
<tr>
<td>EBNA-3A</td>
<td>Nuclear</td>
<td>Contributes to the initiation of cellular proliferation in EBV infected B cells</td>
</tr>
</tbody>
</table>

The oncogenesis is multistep process and needs additional events at cellular level – higher telomerase regulation, suppression of cell anti-oncogenic proteins such as pRb, p53 and others.

In 80% of cases of Burkitt’s lymphoma, a chromosomal translocation (t8:14) in the long arm c-myc region is found. This leads to overexpression of c-myc and uncontrolled proliferation of B-lymphocytes. Another factor in this process is the loss of host immune control – a fact that explains the linkage between lymphoma and malaria (27).

Geographic and genetic factors play important role in the pathogenicity of nasopharyngeal carcinoma (NPC), too. There is a positive association between NPC and HLA alleles A2, B14 and B46, responsible for chromosomal aberrations-(1:3) and gene polymorphism (41-43).
The main reason for post-transplant lymphoproliferative disorders (PTLD) is the impossibility of CTL to control EBV infected B-lymphocytes resulting in their uncontrolled spread and proliferation.

**CONCLUSIONS**

The wide presence of EBV and its association with several diseases explains its high social importance. Studying EBV pathogenesis helps understand the disease genesis and will help future therapeutics and prevention.

**REFERENCES**

22. Rowe M, Lear AL, Croom-Carter D, Davies AH, Rickinson AB. Three pathways of Epstein-Barr virus...


