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ORIGINAL ARTICLE

A brief overview of the Epstein Barr virus and its association with Burkitt's lymphoma

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Abstract: *Epstein Barr virus (EBV) is known as an oncovirus and associates with several human malignancies such as Burkitt's lymphoma, other non-Hodgkin lymphomas, nasopharyngeal carcinoma, Hodgkin's disease, gastric adenocarcinoma, etc. in Burkitt's lymphoma, and the key event is the translocation of MYC gene, that increase of cell survival and aberrant expression of MYC gene. The biology of EBV and its function in the development of Burkitt's lymphoma are discussed in this review.*

Keywords: *Epstein Barr virus, oncovirus, Burkitt's lymphoma, MYC gene*

INTRODUCTION

Epstein–Barr virus (EBV), discovered in 1964 by Epstein, Achong, and Barr from cultured cells of Burkitt's lymphoma by electron microscopy [1]. The EBV is recognized as the first human tumor virus. It is associated with lymphomas, epithelial cell cancers, nasopharyngeal carcinoma, and gastric cancer [2, 3]. EBV infection is mainly transmitted by saliva. The prevalence of EBV in the worldwide population is more than 90%. Most of the EBV carrier individuals do not have any symptoms. However, it is estimated that EBV has a role in the development of approximately 200,000 new cancer cases worldwide each year. Also, it is responsible for almost 2% of all human cancers, and according to current evidence, EBV may be a co-factor in the development of other malignancies, such as breast and cervical cancer [4].

EBV virion structure

EBV (also knowns as human gammaherpesvirus 4) is a member of the Herpesviridae family and a common virus among humans. EBV has a linear, double-stranded DNA genome with a length of 184 kb in a nucleocapsid with 162 capsomeres, which is encapsulated in an envelope with external glycoprotein spikes. The virion diameter is roughly 120-180 nm [5, 6].

Replication cycle

EBV infection targets epithelial and B-cells. Eight glycoproteins are involved in virus entry depends on the type of cell (Table 1) [7]. One of the most abundant glycoproteins of EBV called GP350\220 is responsible for the binding to B-cells, in which it binds to complement receptor type 2 (CR2) on B-cells with high affinity [8]. Although this is a key step for virus attachment to the target cells, other factors also are required for infecting target cells. Major histocompatibility complex class II (MHC-II) on B-cell surface act as a co-factor that interacts with the three viral glycoprotein gp42, gL, and gH to bind the virus to B-cell [9-11].

The fusion of the viral envelope with the cell membrane begins with the interplay of gp42 and HLA class II that eventually leads to endocytosis of the virus into the cell [11]. Complement receptor 1 (also known as CD35) (CR1) is an additional adhesion factor that binds to gp350\220 and can provide a pathway for EBV entry into CD21 negative cells such as immature B-cell. Furthermore, CD35 is sufficient for EBV binding, MHC-II expression is also needed for cell entry and infection. The gp350 mediates the attachment of EBV to epithelial cells, however, it can be fulfilled by gHgL complex on CR2 negative cells [20]. The gHgL complex binds specifically to epithelial cells, not B-cells, and the gH-null virus loses the ability to link to a CR2-negative epithelial cell but still can use gp350 to bind to CR2-positive cells [21-23]. The ability of gHgL to bind to CR2 negative epithelial cells can be blocked by the presence of gp42 [21]. It also prevents the virus's ability to infect CR2-positive cells [24]. In epithelial

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cells lacking CD21, the BMRF-2 protein of the virus interacts with cellular β1 integrin, and gHgL proteins interact with αvβ6\8 integrin to initiate virus fusion [25]. Endocytosis of the EBV into the vesicle and fusion of the virus with the vesicle membrane results in the release of the nucleocapsid into the cytoplasm. As the nucleocapsid dissolves, the genome is transferred to the nucleus [26].

Table 1: Roles of envelope glycoproteins in entry to epithelial and B-cells

Gene	Glycoprotein	Role in virus entry	REF
BLLF1	Gp350	Binding to CR2\CD21 receptor in B cell	[12]
BXLF2	gH	Attachment of virus to epithelial cells, also essential for penetration to B-cells	[13]
BKRF2	gL	Heterodimer gH\gL is critical for entry into the host cell	[14]
BZLF2	gp42	Acting as co-receptor for fusion and entry of EBV into the B-cell	[15]
BALF4	gB	Required for fusion with both epithelial cell and B-cell	[16]
BLRF1	gN	It may play a role in post-fusion events	[17]
BBRF3	gM	Co-expressed with gN	[18]
BMRF2	BMRF2	Binding to host beta 1 integrin family and facilitates virus attachment to the epithelial cell	[19]

Lytic replication

Lytic replication leads to the production of infectious viruses. Lytic replication can occur in both B-cell and epithelial cells. The frequent replication cycle in B-cell occurs after reactivation from latency, while the replication cycle in epithelial cells initiates after entry of the virus [27]. For lytic replication of the viral life cycle, its genome must be linear [28]. In the latent phase, the genome is circular, so it should be linearized in the process of lytic reactivation [28]. During lytic replication, the viral DNA polymerase is responsible for the replication of the viral genome, but in the latent phase, it uses the host cell DNA polymerase to replicate its genome [26]. Three consecutive steps are involved in the production of lytic genes: intermediate early (IE), early (E), and late [29]. In the IE step, two essential genes BZLF1 and BRLF1, are expressed.

These genes act as trans-activators in activating the virus and initiating the cascade of viral genes expression [26]. Expression of the IE BZLF1 gene can induce a switch from latent to lytic infection. BZLF1 binds to the promoters of E genes and encodes proteins that are crucial for viral DNA replication. It recruits RNA polymerase II on their promoters and inducing the lytic cascade mechanism [29].

Expression of one of the BZLF1 or BRLF1 genes is enough to induce lytic replication in both epithelial cells and B-cells. The expression of one IE protein causes the expression of other proteins [30]. Early lytic gene products include a wide range of activities such as replication, metabolism, and blockade of antigen processing [31]. BNLF2a is one of the EBV viral genes expressed in the early lytic stage that plays an important role in escaping the virus from the immune system by expression of a 60 amino acid protein that interferes with antigen presentation to CD8+ cells [32-34]. Late lytic gene products often have a structural role, such as viral capsid antigen (VCA), which is involved in the production of the viral capsid. Several late lytic gene products such as BCRF1 are involved in escaping the virus from the immune system [35].

Latency

Unlike the lytic phase, progeny virions are not produced in the chronic phase, and it is a persistent viral infection. EBV persistent infection is mainly seen in memory B-cells but is also found in epithelial cells [36]. Actually, it is thought that one in the millions of B-cells can carry the virus genome as an episome or integrated DNA after recovering from an acute infection [6, 37].

During the latent phase, episome replication occurs by the host DNA polymerase. In the latency phase, only a limited portion of the genes are expressed [38]. These genes contain five EBV-encoded nuclear antigens (EBNA), two latent membrane proteins (LMP), EBV-encoded small RNA (EBER), and BART RNA (Table 2) [39, 40]. Three patterns of expression of latency genes exist that which of them have different expression patterns and are known as latency programs. Latency programs include latency I, latency II and latency III that each of which can lead to the expression of specific viral genes [41, 42]. Three latency program patterns have been determined in different Burkitt lymphoma and lymphoblastoid cell lines (LCL) and are reported in Table 3 [36].

Table 2: expression of Epstein-Barr virus latent genes

Latent gene	Roles	REF
EBNA-1	Responsible for DNA replication, mitotic segregation, EBV transcriptional activation, autoregulation	[36]
EBNA-2	Main viral trans-activator, critical for B-cell activation	[37]
EBNA-LP	Co-operate with EBNA-2 in viral and cellular gene transcription	[38]
EBNA-3A	With EBNA-3C block EBNA-2 by interaction with RBPG proteins, decrease expression of MYC	[39]
EBNA-3B	It is essential for B-cell transformation, tumor suppressor	[40]
EBNA-3C	Essential for efficient B-cell transformation, also like EBNA-2, LP and EBNA-3C regulates viral and cellular gene expression	[41]
LMP-1	B-cell and epithelial cell transformation, critical role as the primary EBV-encoded oncogene, and active NF-KB and P38 pathway, immune regulation	[42]
LMP-2A	Play a key role in improving cell adhesion, cell motility, and inhibition of epithelial cells also, can activation signaling pathways such as the PI3K\AKT, NF-KB, B-Catenin, STAT, and syk tyrosine kinase and immune modulation	[43]
LMP-2B	-	[43]
EBER	Play a key role in modulating innate immunity response, cell resistance to PKR-mediated apoptosis	[44]
BART	Immune regulation, cancer progression	[45]

Table 3: Epstein-Barr virus latency pattern

Gene expressed	EBNA-1	EBNA-2	EBNA-3A	EBNA-3B	EBNA-3C	EBNA-LP	LMP1	LMP-2A	LMP-2B	EBER	BART
Latency I	+	-	-	-	-	-	-	+	+	+	+
Latency II	+	-	-	-	-	+	+	+	+	+	+
Latency III	+	+	+	+	+	+	+	+	+	+	+

Epstein-Barr Virus microRNAs

The first human viral-encoded miRNAs were discovered in EBV in 2004 by Pfeffer et al. [43]. microRNAs (miRNAs) are small non-coding RNA containing about 20-22 nucleotides that play a crucial role in regulating gene expression [44]. For cell-to-cell transmission, miRNAs can be packaged into exosomes and released into the extracellular space [45]. various cell types can secreted exosomes into body fluids, including blood, urine, saliva, breast milk, cerebrospinal fluid, ascites, etc [46] miRNA plays an important role in the pathogenesis of EBV-associated cancers.

EBV encodes over 40 miRNAs, which are derived from approximately 25 viral-miRNA precursors [47]. Forty of them are located to BamHI-A rightward transcript (BART), and four are located to the BamHI fragment H rightward open reading frame 1 (BHRF1) [48]. These miRNAs have distinct levels of expression at different phases of latency. EBV miRNAs can modulate infection and pathogenesis by regulating the expression of viral genes [49]. The miRNA can be effective in escaping the EBV from the immune system by suppressing the release of pro-inflammatory cytokines, such as IL-12, and repress the differentiation of CD4+ T cells. miRNA directly targets the transported TAP2 and decreases levels of TAP1 and MHC class 1 molecule, which can cause the EBV-infected cells to evade CD8+ T cells [50].

Epidemiology

In developing countries, the infection occurs in children (in the first years of life), whereas in developed countries, it is usually delayed and seen in adolescence [51]. In some developed countries, however, the rate of infection is bimodal, with peaks in children under five years and again after ten years [52]. The cause of this bimodality is the transmission of the virus through oral excretion between parents and infants and from partners in adults [53]. The EBV antibody titer in seropositive individuals shows a U-shaped pattern, and the highest antibody titer is seen in infants and the elderly [54]. The high antibody titer in children is usually a reflection of primary infection and, in the elderly, is due to a reduced immune response [55, 56]. EBV has spread worldwide but with remarkable geographical variety in the distribution of EBV genotypes [57]. EBV contains two major types, I and II, that differ in the genes expressed in latently infected cells [58]. Both types are detectable all over the world, but type I is more common.

However, in some areas such as Africa, Papua New Guinea, and Alaska the second type is the most common [59, 60]. The prevalence of the virus is not significantly different between men and women, but overall, antibody titers are higher in women than men [53]. EBV is also associated with socioeconomic conditions. Poor socioeconomic status is related to primary EBV infections, whereas late primary EBV

infection occurs more frequently in people with higher socioeconomic status [61].

Transmission

EBV usually is transmitted through oropharyngeal secretions. In adults and adolescents, the primary cause of virus transmission is kissing, whereas saliva on stuff such as toys or fingers is the primary cause of transmission among children. EBV is detectable in cervical secretions of 8-28% of young girls and adult women and also in the seminal fluid of men. In rare circumstances, the virus can be transmitted through breast milk. It can also be transmitted through blood transfusion and transplanted allogeneic hematopoietic cells or solid donor organs [53, 62].

Vaccine for EBV

Vaccination is the most effective way to avoid contracting EBV. More than 90% of the world's human population is infected by EBV, therefore, the vaccine is greatly important. The evidence shows that the EBV vaccine can prevent more than 200,000 new malignancies per year worldwide. gp350 viral antigen is the most abundant glycoproteins on the surface of EBV that known as the first candidate for the vaccine. gp350 has an important role in the efficient infection, although the gp350 deficient virions can still infect B cell, gp350 antibodies predominantly neutralize B cell infection. The amino-acid sequence of gp350 is extremely conserved among diverse strains, especially in the amino-terminal region, and 97% amino-acid similarity between EBV type1 and type 2 [63]. Among all EBV glycoproteins, gp350 is a promising vaccine target to block b cell infection and is also a target for T cell-mediated immunity (64-66). gp350 in combination with the AS04 adjuvant demonstrated can prevent the development of infectious mononucleosis (IM) after EBV infection [67]. Monoclonal antibodies against gp42 can inhibit EBV infectivity of B cells, while infection of epithelial cells inhibited by a monoclonal antibody against gH\gL or BMFR2 antigens [68, 69]. EBV neutralizing antibody titers correlate more with gp350 antibody compared with antibody titers to gp42 [70]. Furthermore, the latent antigen EBNA3A with tetanus toxoid formulated in water-in-oil adjuvant acts as a vaccine that is designated to induce EBV-specific T cell response. In almost 90% of vaccines, CD8 T-cell responses are obvious but can not prevent EBV infection. Although it seems that vaccination can reduce the incidence of IM [71].

Diagnosis

There is a spectrum of laboratory approaches for the diagnosis of EBV infection. The strategy of diagnosis is different between immunocompromised and immunocompetent individuals because therapeutic interventions in these groups are different. The diagnosis of primary infection is based on antibody assays [72]. Three serological patterns such as immunoglobulin (Ig) G and M antibodies to viral capsid antigens (VCA) and IgG antibodies to EBNA1 are a minimal requirement for detection of primary EBV infection. Immunofluorescence assay (IFA) is a gold standard for the diagnosis of EBV infection [73]. enzyme immunoassay (EIA) and chemiluminescence immunoassay (CLIA) are often performed as screening tests, while western blot analysis is used for confirmation [74]. For detecting and quantifying the virus in body fluids, polymerase chain reaction (PCR) analysis is the best approach, and tissue samples as well as, it can be used for measuring the viral load [75]. Additionally, the detection of EBV-encoded RNA transcripts (EBERs) by in situ hybridization is the gold standard for finding EBV in tissue [76].

Treatment

For the treatment of EBV infection, there is no approved antiviral medication. More physicians prescribe acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) to alleviate fever and pain [77]. Fluids and nutrition are highly recommended, especially for febrile cases. Since most patients have anorexia in the first and seconds weeks, nutrition is very important [78]. Several antiviral drugs are used for EBV infection, although non specifically against EBV, and most of these drugs are nucleoside-analogue [79]. The only class of drugs that assess for EBV infection in clinical trials is nucleoside-like agents. The nucleoside analogue in the drug disrupts the synthesis of viral DNA by inhibiting viral DNA polymerase. Acyclovir is a nucleoside analogue that can reduce EBV shedding but without evidence of clinical benefit [80]. Studies have also reported the use of other agents, such as interleukin 2 [81], interferon-alpha [82], and Intravenous immunoglobulins (IVIg) [83] in the management of EBV related diseases.

EBV AND BURKITT LYMPHOMA

For the first time, Burkitt lymphoma (BL) was described 60 years ago by Denis Burkitt as a disorder with jaw tumors in African children, which he called “African lymphoma” [84, 85]. BL is an aggressive B-cell malignancy among non-Hodgkin lymphoma (NHL) types [86, 87]. It is divided into three classes, which vary in geographic distribution and relation with EBV: endemic (eBL), sporadic (sBL), and HIV-associated BL [85, 88].

• **Endemic BL** (eBL) is one of the most common childhood cancers in Equatorial Africa and is accounts for 75% of malignant disorders in children aged 4-7 years [89]. Evidence

suggested a supportive relationship between EBV and eBL, which almost all eBL cases are associated with EBV [90]. Plasmodium falciparum contributes to the etiology of eBL first by disrupting immune response against EBV, thereby increase virus replication, second by stimulating B-cell activation, which leads to EBV lytic reactivation and abnormal expression of activation-induced cytidine deaminase (AID) that elevate the probability of MYC translocation triggering oncogenesis [91]. Jaw and kidney involvement are frequent, as well as the central nervous system (CNS), ileal, cecal, gonads, facial bone, thyroid, and breast can be affected [92].

• **Sporadic BL** (sBL) is almost occurring worldwide, without an association between geographic and climatic conditions. sBL is responsible for 1-2% of adult lymphoma, up to 40% of children's lymphoma in western Europe and the United States. The abdomen, particularly the ileocecal region and lymph node, are the most common site of involvement. sBL is associated with EBV infection, ranging from 10-30% positivity in various regions [93].

• **HIV-associated BL** mainly occurs in human immune-deficiency virus (HIV) infection. HIV expansion in developed and developing countries has increased EBV-dependent BL in adult populations and is an early marker for AIDS progression in HIV-positive individuals [94]. EBV is observed in 30-40% of HIV-associated BL cases (Table 4) [95].

Table 4: Characteristic features of the various Burkitt lymphoma subtypes

	Endemic BL	Sporadic BL	HIV-associated BL
Geographical distribution	Equatorial Africa And Papua New Guinea	Worldwide	Worldwide
Incidence	5-15\10^5	1-2\10^6	Variable
Age	4-7 years	All ages	All ages
EBV-association	>90%	10-30%	30-40%
Co-factor	EBV, malaria	Unknown	HIV
Location of MYC breakpoint	More than 1 Kb upstream of first exon	Exon1 the MYC gene	Exon1 the MYC gene
Location of Ig breakpoint	Joining (j) region, Sμ switch region of Ig	Sμ, sα and j region	Sμ
Tumor site	Most common in the jaw, it can also occur in the abdomen, kidney, and ovaries	Most common in the abdomen, it can also occur in the kidney	Lymph node, bone marrow, abdomen, and CNS

The hallmark feature of all BL is the translocation of the MYC gene, which results in the upregulation of MYC protein. Most frequent translocation (approximately 80% of cases) between the MYC gene with a heavy chain of immunoglobulin (14q32). The two other translocations can be found in BL, translocation of c-MYC with the light chain of immunoglobulin at 2p12 and 22q11 [96, 97]. The c-MYC has essential roles in different cellular processes, such as regulates the transcription of various genes, cell growth, signal transduction, regulation of RNA processing, immune function, and apoptosis. MYC overexpression does not alone adequate to initiate lymphomagenesis. Dysregulation of microRNAs (miRNA) may lead to increase expression of MYC independent of chromosomal translocation or gene amplification [98].

Furthermore, in sporadic Burkitt's lymphoma, additional mutations related to the MYC gene have been discovered. Mutations in TCF3 or ID3, which is a protein that blocks TCF3 activity, are observed in about 70% of cases. In addition, almost one-third of sBL cases have a mutation in CCND3, which encodes cyclin D3 [99, 100]. sBL mostly causes abdominal tumors, while eBL is often present in the jaw and kidney but can also be present in the abdomen, ovaries, facial bone, and other extranodal sites [101, 102].

Burkitt's tumors have historically been monomorphic medium-sized cells with rounded nuclei, many nucleoli, and copious basophilic cytoplasm. Due to the presence of high numbers of macrophages, Tumours show a ''starry sky'' pattern [103]. The proliferation of BL cells is rapid, and their doubling time is 24-48 hours [104]. Their rate of apoptosis is also high. BL cells usually express B-cell markers including CD19, CD20, CD22, and IgM and germinal center markers such as CD10, BCL6, and human germinal center associated lymphoma (HGAL) protein [105]. The cell surface phenotype of BL cells reflects the origin of the germinal center, whereas jaw and ovaries are the primary sites of tumor growth, do not usually contain germinal center [106]. It seems that translocation of the MYC gene to immunoglobulin chains in eBL occurs during the VDJ recombination [107, 108]. This process occurs by a VDJ recombinase activating an enzyme called RAG-1/2 expressed in both pre-B and germinal center B cells [109]. However, in sBL and HIV associated, this

chromosomal breakage happens mainly in the class switch region [110]. The GC involvement is an essential factor in the pathogenesis of this disease, whether in MYC translocation or association with EBV, malaria, and HIV infection as a co-factor [111]. Malaria and HIV infection, for example, has been determined to activate B cells [112]. Evidence from clonal EBV genomes in Burkitt's lymphoma tissues showing that the tumor is originated from a single infected cell. EBV gene products can directly aid cell survival in the BL cells. Both EBNA-1 and the EBERs, for example, have a function in BL cell survival and apoptosis prevention.

CONCLUSION

Burkitt's lymphoma is known as a highly aggressive non-hodgkin lymphoma with a diverse pattern of clinical presentation. It is a multi-factorial disease, although MYC translocation accounts for one of the main causes of its development. Other risk factors contribute significantly to MYC translocation. Finding the detailed role of each risk factor in the occurrence and progression of Burkitt's lymphoma is a good area in medical research. A better understanding of the detailed mechanism of Burkitt's lymphoma pathogenesis, as well as the precise role of each factor, can lead to the introduction of more effective prevention and therapy strategies.

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Competing interests

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