



# Molecular Characterization of the Epstein-Barr Virus *BGLF2* Gene, its Expression, and Subcellular Localization

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**Background:** Epstein-Barr virus (EBV) is a universal herpes virus which can cause a life-long and largely asymptomatic infection in the human population. However, the exact pathogenesis of the EBV infection is not well known.

**Objective:** A comprehensive bioinformatics prediction was carried out for investigating the molecular properties of the *BGLF2* and to afford a foundation for future research of the role and instrument of *BGLF2* in the course of EBV infection.

**Materials and Methods:** A 1011-base-pair sequence of *BGLF2* gene from the Epstein-Barr virus (EBV) Akata strain genome was amplified using polymerase chain reaction and was further characterized by cloning, sequencing, and subcellular localization in the COS-7 cells.

**Results:** The bioinformatics analysis demonstrated that EBV *BGLF2* gene encodes a putative *BGLF2* polypeptide which contains a conservative Herpes\_UL16 domain. It was established that the polypeptide shows a close relationship with the Herpes UL16 tegument protein family and is extremely conserved among its homologues proteins encoded by *UL16* genes. Multiple sequence alignments of the nucleic acid and amino acid sequence showed that the gene product of EBV *BGLF2* contains a comparatively higher homology with the *BGLF2*-like proteins of the subfamily *Gammaherpesvirinae* than that of other subfamilies of the herpes virus. Moreover, the phylogenetic analyses suggested that EBV *BGLF2* has a close genetic relationship with the member of *Gammaherpesvirinae*; in particular with the members of *Cercopithecine herpesvirus 15* and *Callitrichine herpesvirus 3*. An antigen epitope analysis indicated that *BGLF2* contains several potential B-cell epitopes. In addition, the secondary structure, as well as the three dimensional structure prediction suggests that *BGLF2* consists of the both  $\alpha$ -helix and  $\beta$ -strand. Besides, the subcellular localization prediction revealed that *BGLF2* localizes in both nucleus and cytoplasm.

**Conclusions:** Illustrating the relevance of the molecular properties and genetic evolution of EBV, *BGLF2* will offer the perspectives for further study on the role and mechanism of the *BGLF2* in course of EBV infection. These works will also conduct our understanding of the EBV at the molecular level as well as enriching the herpesvirus database.

**Keywords:** *BGLF2*, Cloning, Bioinformatics analysis, Epstein-Barr virus, Molecular property

## 1. Background

Epstein-Barr virus (EBV), also named as human herpesvirus 4 (HHV-4), is a ubiquitous herpesvirus with the property of being universally spread in the human population, inducing a perpetual, and to a large extent asymptomatic infection. It was shown that EBV is associated with the infectious mononucleosis and various

malignancies, including Burkitt Lymphoma (BL), Hodgkin Lymphoma (HL), post-transplant lymphoproliferative disease (PTLD), as well as an epithelial tumour, T/NK cell lymphomas, and nasopharyngeal carcinoma (NPC) (1-3). However, the exact pathogenic mechanism of the EBV infection is still less understood.

As a potential tegument protein, EBV *BGLF2* is

important for re-envelopment in the cytoplasm or plasma membrane, which can be detected in the mature EBV virions. BGLF2 is also related to EBV reactivation via stimulation of p38 mitogen-activated protein kinase (1-3). However, the accurate functions of *BGLF2* gene and its product BGLF2 are not well known.

## 2. Objectives

For sake of deciphering the role of BGLF2, the *BGLF2* gene was amplified from the DNA template of the EBV Akata strain by employing polymerase chain reaction (PCR) followed by cloning, sequencing, and expressing in the COS-7 cells. Subsequently, a comprehensive bioinformatics prediction was carried out to investigate the molecular properties of the *BGLF2* and to offer a foundation for future investigation of the role and the mechanism of BGLF2 in the course of EBV infection. The prediction included various bioinformatics tools such as clone manager professional suite 8.0, Conserved Domains, SignalP-4.0, DNASTar 7.0, NetPhos 2.0, Bioedit 7.0, PSIPred, and CPHmodels 3.2.

## 3. Materials and Methods

### 3.1. Cloning and Sequencing of EBV BGLF2

The primers for PCR amplification of EBV Akata strain *BGLF2* (accession No. KC207813) were designed using clone manager professional suite 8.0 and afforded by Sangon biotech (Shanghai, China). The forward primer BGLF2-F: 5'-TTGAATTCCATGGCATCCGCCGCGAACAG-3' is complementary to the initial 20 nucleotides of *BGLF2* and introduced with an *EcoRI* restriction site (underlined) for cloning. The reverse primer BGLF2-R: 5'-AAGGATCCTCAATAAGAATGTAAGACCTGACG-3' anneals to the 23 terminal nucleotides of *BGLF2* and introduced with a *BamHI* restriction site (underlined).

The *BGLF2* gene was amplified with KOD-plus-Neo DNA polymerase (TOYOBO) via PCR using the EBV-BAC (bacterial artificial chromosome) of Akata strain (AK-BAC) (a generous gift from Dr. Teru Kanda) as the template DNA (4). PCR profiles implicated initial pre-denaturation for 10 min at 94 °C followed by 30 cycles of denaturation at 95 °C for 50 s, annealing at 54 °C for 45 s, and extension at 72 °C for 70 s. The final extension step was implemented at 72 °C for 10 min. Then, the PCR product was purified and digested with *EcoRI* and *BamHI* and inserted into the corresponding digested eucaryotic yellow fluorescent protein expression vector pEYFP-C1 (Clontech, USA) to generate pEYFP-BGLF2. The existence of the proper fragment in the attained plasmid was then verified by the restriction analysis, PCR, and sequencing (5).

### 3.2. Western Blot Analysis

To further verify the validity of pEYFP-BGLF2, COS-7 cells were seeded at  $2.5 \times 10^5$  cells per well in a 12-well plate and at 80% confluence after overnight incubation cells were transiently transfected with the expressing plasmid pEYFP-BGLF2 or empty vector pEYFP-C1 by the standard calcium phosphate precipitation process. The transfection efficiencies of the different plasmids in COS-7 cells were evaluated by enhanced yellow fluorescent protein (EYFP). Twenty-four hours later the transfected cells were harvested and fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and subsequent Western blot analysis was carried out as described previously using monoclonal antibody (mAb) against YFP (Santa Cruz Biotechnology, Santa Cruz, CA) (6-12).

### 3.3. Plasmid Transfection and Fluorescence Microscopy

Transfection and fluorescence microscopy procedures were carried out as reported in our previous researches (6-8, 13-15). For duplicate the assay, each transfection was manipulated at least three times. Results presented are from one representative assay. Results were analyzed by a Zeiss Axiovert 200 M microscope (Germany). All the photomicrographs were captured at an enlargement of  $\times 400$ . Each picture shows an enormous majority of the cells with analogous subcellular localizations. The light-translucent images indicate cellular morphology. Fluorescence pictures of the EYFP tagged fusion proteins are indicated in pseudocolor green. Figures were refined with Adobe Photoshop software.

### 3.4. Nucleotide Sequence Bioinformatics Analysis of EBV BGLF2

To determine the nucleotide sequence similarity, the National Center for Biotechnology Information (NCBI) nucleotide Basic Local Alignment Search Tool (16) was applied. Subsequently, Clustal V in the MegAlign program of DNASTar (version 7.0, DNASTar, Inc.) was employed to analyze the nucleotide sequence homology of the 55 BGLF2-like proteins of herpesviruses (**Supplementary File**).

### 3.5. Bioinformatics analysis of the Deduced Amino Acid (AA) Sequence of EBV BGLF2

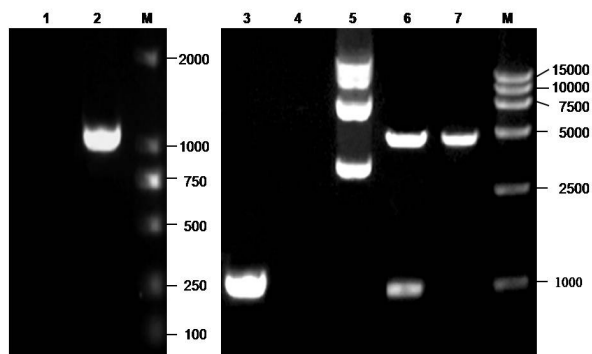
For AA sequence comparison, homology search, and conserved domain analysis, the AA sequence of BGLF2 was analyzed with protein BLAST (16), FASTA (17), and the Conserved Domains search tool (18), respectively. To compare BGLF2 with BGLF2-like proteins of the other herpesviruses (see Supplementary File), we analyzed the AA sequence homology and phylogenetic relationships using DNASTar 7.0. To predict transmembrane domain, the signal peptide sequence, glycosylation site,

phosphorylation site, hydrophobic and hydrophilic regions, B-cell epitope, secondary structure, and the 3-dimensional (3D) structure of the BGLF2, TMHMM (19), SignalP-4.0 Server (20), NetNGlyc 1.0 (21), NetPhos 2.0 (22), Bioedit 7.0, DNASTar 7.0, PSIPred (23), and CPHmodels 3.2 (24) were applied, respectively.

## 4. Results

### 4.1. PCR Amplification and Cloning of EBV BGLF2

To get *BGLF2* gene, we performed PCR on the basis of the DNA template from the purified BAC DNA of the EBV Akata strain. As shown in **Figure 1**, no specific band was amplified from the negative control (Fig. 1, lane 1), while a target fragment of 1011 bp, which is consistent with the expected size was amplified from EBV BAC DNA (see Fig. 1, lane 2). The DNA fragment was then ligated into the eukaryotic expression vector pEYFP-C1, transformed into *E. coli*, and characterized by colony PCR (Fig. 1, lane 3 and lane 4). Subsequently, the identified pEYFP-BGLF2 plasmid was extracted from the positive colony (see Fig. 1, lane 5), which was further verified by restriction digestion analysis (Fig. 1, lane 6 and lane 7) and DNA sequencing. Compared to that of the EBV Akata strain, the sequencing result indicated that there was no AA mutation existing in the clone (data not shown).



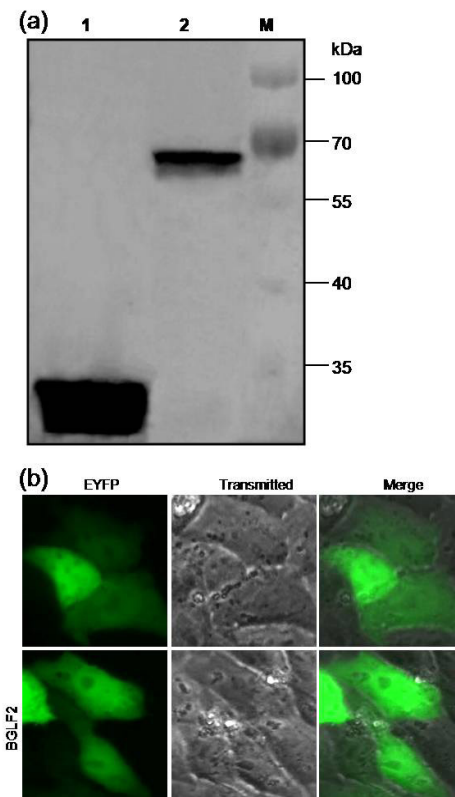
**Figure 1.** Cloning and verification of the plasmid pEYFP-BGLF2. Lane 1 to lane 4, PCR amplification product of no template control (lane 1), Akata-derived EBV-BAC DNA (lane 2), PCR product of pEYFP-BGLF2 (lane 3) or PCR product of the empty vector of pEYFP-C1 (lane 4) colony by primers BGLF2-F and BGLF2-R; lane 5, plasmid pEYFP-BGLF2; lane 6 and lane 7, restriction digestion product (approximately 4700 and 1018 bp, respectively) of the pEYFP-BGLF2 or restriction digestion product (approximately 4700 and 31 bp, respectively) of the pEYFP-C1 with EcoRI and BamHI. Lane M, DNA marker (TaKaRa). Samples were electrophoresed using a 1% agarose gel and stained with ethidium bromide.

### 4.2. Expression of EBV BGLF2 in Eukaryotic Cells

After sequence confirmation, the recombinant plasmid pEYFP-BGLF2 and the empty vector pEYFP-C1 were transfected into the expression host; the COS-7 cells. After 24 hours, the expression of EYFP-BGLF2 and EYFP were examined via Western blot using anti-YFP mAb. As a result a distinct band of approximately 66 kDa molecular weight, corresponding to the expected size of EYFP-tagged BGLF2 fusion protein (**Fig. 2a**), was detected. However, a band with only about 27 kDa was observed in the control COS-7 cells transfected with pEYFP-C1 (Fig. 2a). This result assured that the open reading frame of the BGLF2 was properly expressed in the transfected COS-7 Cells

### 4.3. Subcellular Localization of BGLF2 in the Transfected Cells

It's well known that determination of the subcellular localization is one way to evaluate the potential roles



**Figure 2.** Protein expression and subcellular localization of the EBV BGLF2 in transfected cells. (a) Expression analysis of the EBV BGLF2 in transfected cells using anti-YFP mAb through Western blot. Lane 1, COS-7 cells transfected with plasmid pEYFP-C1; lane 2, COS-7 cells transfected with plasmid pEYFP-BGLF2; lane M, protein marker (Thermo Scientific PageRuler Prestained Protein Ladder). (b) The subcellular distribution of EYFP and EYFP-BGLF2. Each fluorescence image is representative of the vast majority of the observed cells.

**Table 1.** Multiple nucleic acid sequence and amino acid sequence alignments of EBV *BGLF2* gene with its reference species .

Virus name	AIHV-1	AnHV-1	AoHV-1	AtHV-3	BBHV	BoHV-1	BoHV-4	BoHV-5	CalHV-3	CavHV-2	CeHV-1
PNH <sup>a</sup> (%)	35.3	23.2	25.1	36.8	22.8	24.4	38.7	25.4	54.5	21.9	22.7
PAH <sup>b</sup> (%)	32.5	13.4	14.0	36.7	16.7	15.2	38.6	16.4	60.4	16.5	15.2
Virus name	CeHV-2	CeHV-5	CeHV-9	CeHV-15	CeHV-16	CeHV-17	CMCMV	EEHV	EHV-1	EHV-2	EHV-4
PNH (%)	22.9	23.8	20.2	82.2	23.7	34.8	21.4	22.1	22.3	39.4	22.7
PAH (%)	14.6	16.4	14.3	87.8	14.3	35.1	15.8	16.1	13.4	35.1	13.7
Virus name	EHV-8	EHV-9	FeHV-1	GaHV-3	HHV-1	HHV-3	HHV-5	HHV-6	HHV-7	HHV-8	McHV-3
PNH (%)	22.1	21.7	22.4	22.1	23.1	22.8	22.5	20.8	21.5	35.2	22.8
PAH (%)	13.4	13.1	14.6	14.3	13.7	12.5	14.3	15.2	16.1	30.8	16.1
Virus name	MFRV	MuHV-1	MuHV-2	MuHV-4	MuHV-8	OvHV-2	PLHV-1	PLHV-2	PLHV-3	PnHV-2	RHVP
PNH (%)	35.8	23.9	20.3	32.9	24.1	39.9	42.4	42.8	42.5	21.7	35.2
PAH (%)	34.8	15.2	14.9	29.4	16.1	36.0	42.3	42.6	42.6	14.3	28.7
Virus name	SaHV-1	SaHV-2	SaHV-3	TuHV-1	WMHV	HHV-4 AG876	HHV-4 HKNPC1	HHV-4 GD2	HHV-4 B95-8	HHV-4 GD1	
PNH (%)	22.0	39.1	20.8	24.0	33.4	99.5	99.8	99.8	99.9	100	
PAH (%)	15.2	39.1	16.4	15.8	29.4	100	100	100	100	100	

<sup>a</sup> PNH indicates the multiple nucleic acid sequence alignment of *BGLF2* gene of EBV Akata strain with its homologous genes of 54 selected species (Table 1) by using the MEGALIGN program in LASERGENE (DNASStar 7.0) with Clustal V Method, and sequence distance was calculated using weight matrix Identity. Gaps had been introduced by the alignment program to maximize the homology.

<sup>b</sup> PAH indicates the multiple aa sequence alignment of *BGLF2* of EBV Akata strain with its homologous proteins of 54 selected species (Table 1) by using the MEGALIGN program in LASERGENE (DNASStar 7.0) with Clustal V Method, and sequence distance was calculated using weight matrix PAM250. Gaps had been introduced by the alignment program to maximize the homology.

of some specific proteins. To further investigate the subcellular distribution of *BGLF2* in transfected live cells, fluorescence microscopy was employed. The plasmid pEYFP-*BGLF2* encoding *BGLF2* fused to the C terminus of EYFP was transfected into COS-7 cells to study the subcellular localization of the *BGLF2* in absence of other viral proteins. As shown in Fig. 2b, the control EYFP fluorescence was evenly distributed throughout the cytoplasm and the nucleus in the cells transfected with pEYFP-C1. Moreover, EYFP-*BGLF2* also exhibited a similar subcellular distribution pattern with that of EYFP (Fig. 2b).

#### 4.4 Bioinformatics Analysis of the EBV *BGLF2*

##### Nucleotide Sequence

The nucleotide sequence similarity search using NCBI BLASTN yielded 5 nucleotide sequences (accession Nos. DQ279927, JQ009376, HQ020558, AJ507799, and AY961628) with strong identity to the EBV Akata strain *BGLF2* (up to 98.5, 99.8, 99.8, 99.9, and 100%, respectively) (see **Table 1** and **Fig. 3**); these accessions were corresponded to the *BGLF2* gene of the EBV AG876, HKNPC1, GD2, B95-8, and GD1 strains, respectively. Multiple alignments of EBV *BGLF2*

with 54 homologous reference herpesviruses revealed a remarkably high similarity of 82.2% and 54.5% with the members of subfamily *Gammaherpesvirinae*, that is Cercopithecine herpesvirus 15 (CeHV-15) and Callitrichine herpesvirus 3 (CalHV-3). However, a relatively low similarity was detected between EBV and other members of the subfamily *Betaherpesvirinae* and *Alphaherpesvirinae* (Table 1 and Fig. 3).

#### 4.5 Bioinformatics Analysis of the EBV *BGLF2*

##### Polypeptide Sequence

An AA sequence similarity search using NCBI BLASTP yielded 5 AA sequences (accession Nos. ABB89265, AFJ06878, AEM00649, CAD53441, and AAY41138) that completely matched with the target sequence of the EBV Akata *BGLF2* (accession No. AFY97879; all reach to 100%), and these sequences corresponded to the *BGLF2* protein of EBV AG876, HKNPC1, GD2, B95-8, and GD1 strains, respectively (Table 1 and **Fig. 4**). In addition, multiple sequence alignments of the *BGLF2* with its homologs in the 54 references for the herpesviruses showed a high similarity of 60.4% or 87.8% between *BGLF2* and its CalHV-3 or CeHV-15 counterparts of subfamily *Gammaherpesvirinae*.





**Figure 3.** The multiple nucleic acid sequence alignments of the EBV BGLF2 gene against its reference species (supplementary file) by using the MEGALIGN program in LASERGENE (DNASStar 7.0) with Clustal V Method, and sequence distance calculated using weight matrix identity. Gaps had been introduced by the alignment program to maximize the homology. (The original file is available at [www.jibiotech.com](http://www.jibiotech.com)).

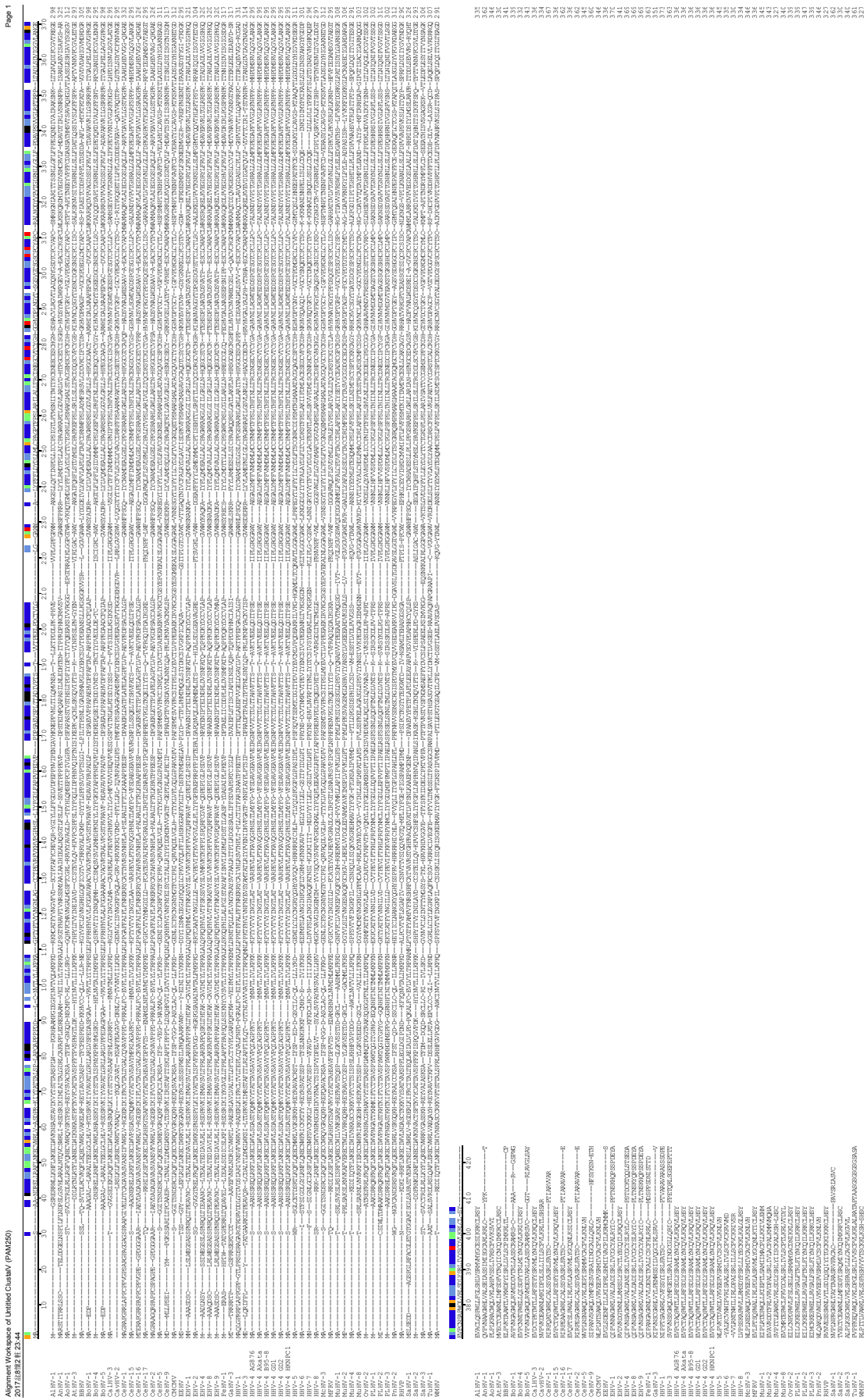
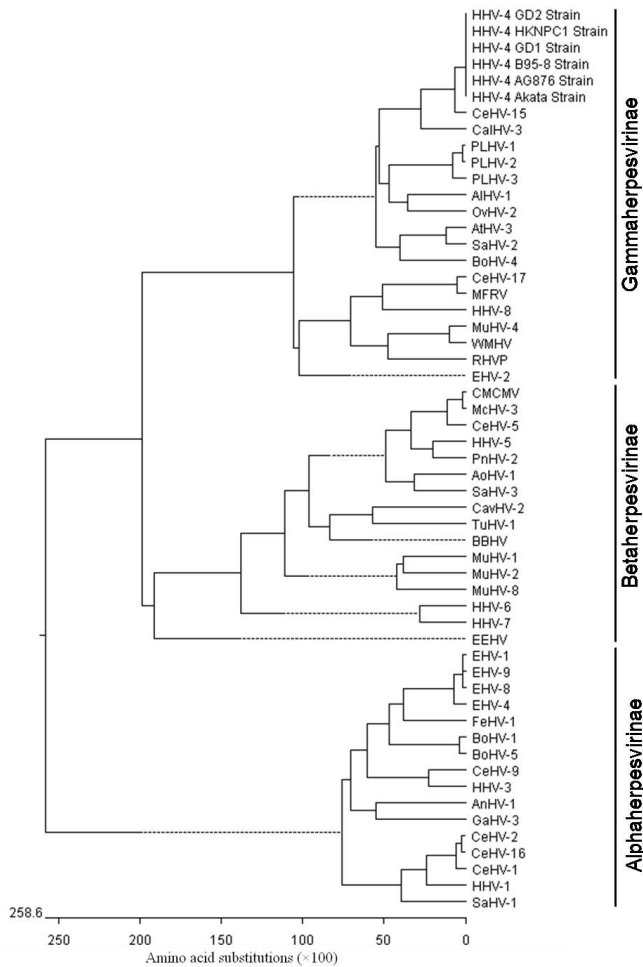


Figure 4. The multiple AA sequence alignments of the EBV BGLF2 gene against its reference species (supplementary file) by using the MEGALIGN program in LASERGENE (DNASar 7.0) with Clustal V Method and sequence distance was calculated using the weight matrix PAM250. Gaps had been introduced by the alignment program to maximize the homology. (The original file is available at www.ijbiotech.com).

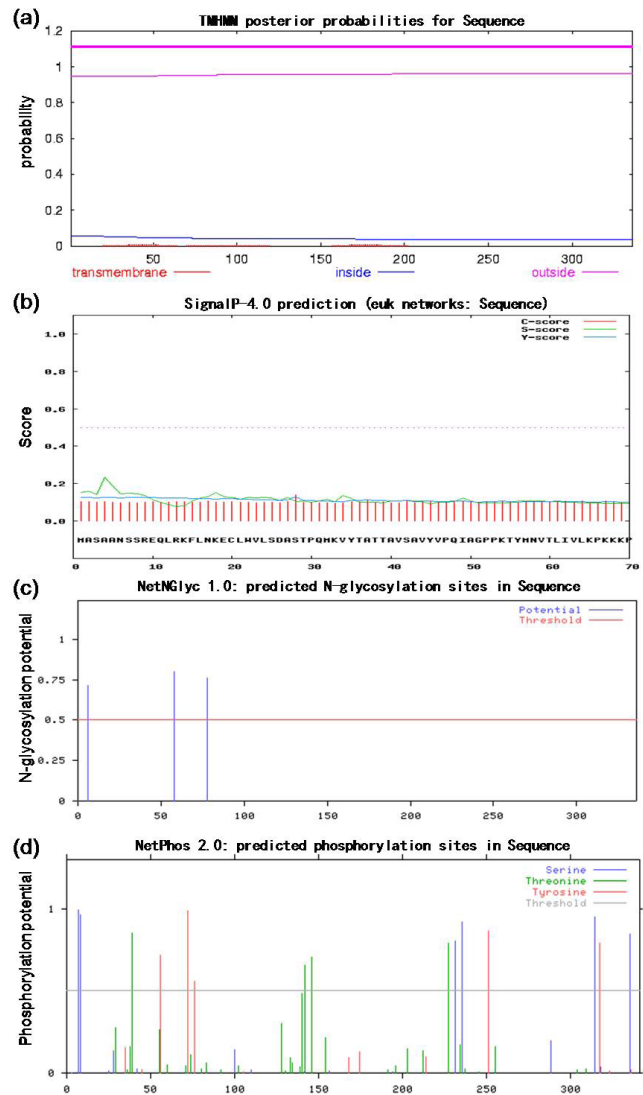


**Figure 5.** The evolutionary relationships of the EBV BGLF2 protein with its 54 reference herpesviruses from different species (see supplementary file). The phylogenetic tree of these proteins was generated using the Megalign program in LASERGENE (DNASTar 7.0) with the Clustal V method and the sequence distance was calculated using weight matrix PAM250. Gaps were introduced by the alignment program to maximize the homology.

However, BGLF2 shared low similarity with the BGLF2-like proteins from the members of the *Betaherpesvirinae* and *Alphaherpesvirinae* subfamilies with values lower than 20% (Table 1 and Fig. 4). The phylogenetic analyses of the EBV and other herpesviruses were performed based on the sequences of BGLF2 and the BGLF2-like proteins of the 54 reference herpesviruses (Table 1). As the results showed, the proteins were preliminarily separated into different subfamilies, i.e. *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae* (see Fig. 5), which is corresponded with the existing classification for the subfamily of herpesvirus. The general branching pattern is also in line with previously published phylogenetic analyses (25, 26). Furthermore, the EBV

Akata, AG876, HKNPC1, GD2, B95-8, and GD1 strains were different from the other Gammaherpesviruses. They clustered together and formed a separate branch and then clustered with members of the subfamily *Gammaherpesvirinae* such as CeHV-15 and CalHV-3. Subsequently, they clustered with other members of the subfamily *Gammaherpesvirinae* (Fig. 5). Therefore, EBV might have a closer evolutionary relationship with the member of subfamily *gammaherpesviruses*.

The transmembrane domain and the signal polypeptide prediction showed no transmembrane domain (Fig. 6a) and the signal polypeptide cleavage site (Fig. 6b) in BGLF2. However, N-linked glycosylation site (Asn-X-Ser/Thr) prediction demonstrated that there are 3

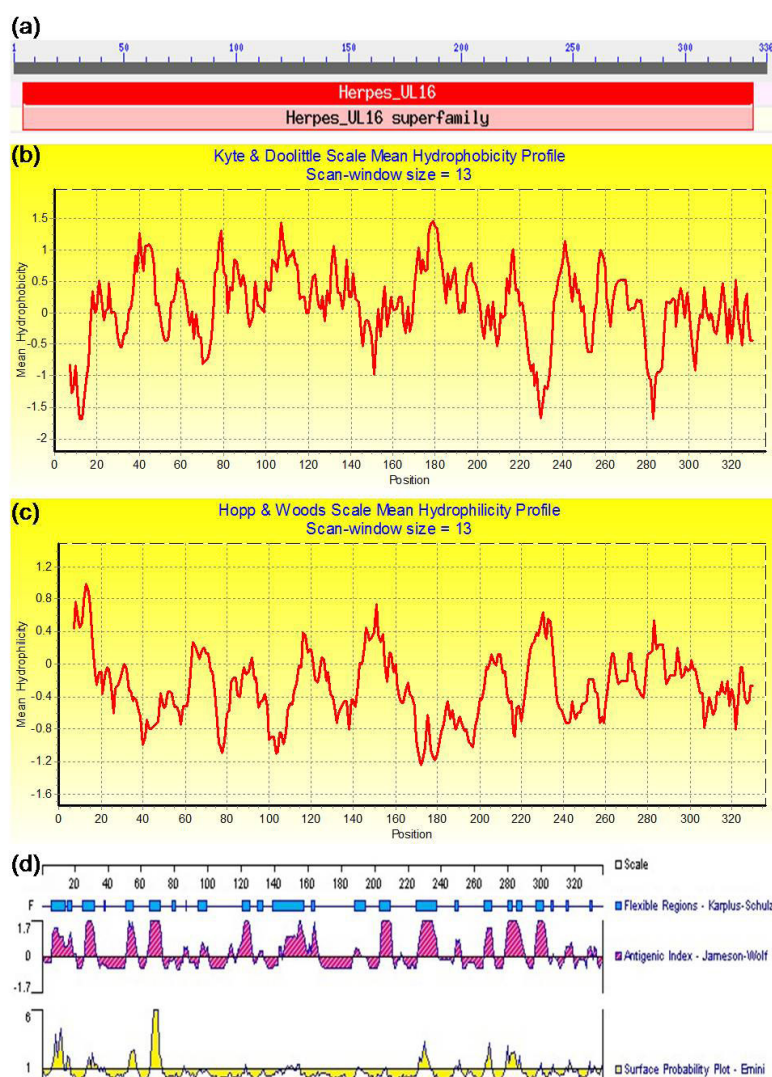


**Figure 6.** The prediction of transmembrane domain, signal peptide sequence, glycosylation site, and phosphorylation site of the EBV BGLF2 using TMHMM program (a), SignalP-4.0 (b), NetNGlyc 1.0 program (c), and NetPhos 2.0 program (d), respectively.

potential N-glycosylation sites in the BGLF2 (Fig. 6c). Interestingly, 15 potential phosphorylation sites were observed in the BGLF2 (Fig. 6d) including 6 serine, 5 tyrosine, and 4 threonine residues.

In addition, the conserved domain analysis indicated that BGLF2 contained an apparently conserved domain of the Herpes\_UL16, which is closely related to the Herpes UL16 tegument protein family (Fig. 7a). The hydrophobicity analysis revealed several potential hydrophobic regions located at the AAs 17-26, 35-48, 56-63, 75-144, 155-164, 168-201, 212-221, 236-278,

289-300, and 314-327, respectively (Fig. 7b). Compared to the hydrophobic region, the proportionality of the hydrophilic region was smaller than the hydrophobic region (Fig. 7c). Furthermore, analysis of a potential B-cell epitope determinant demonstrated several potential B-cell epitopes in the BGLF2 situated in or adjacent to the AAs 7-19, 27-34, 52-57, 65-71, 121-126, 147-165, 204-211, 226-237, 266-272, 277-289, and 296-305, correspondingly (Fig. 7d). Moreover, the secondary structure analysis (Fig. 8a) suggested that the AA sequence of BGLF2 is composed of the random



**Figure 7.** The conserved domain, hydrophobicity, hydrophilicity, and antigenic analyses of the EBV BGLF2. (a) The conserved domain analysis of the BGLF2 using NCBI Conserved Domains search tool. (b) The hydrophobicity or (c) hydrophilicity profile was determined using the values of Kyte and Doolittle (Kyte and Doolittle, 1982) or Hopp and Woods (Hopp and Woods, 1981), respectively, with a 13-amino-acid window. The peaks pointing up represent the most hydrophobic (b) and hydrophilic (c) regions, respectively. (d) Antigenic analysis of the BGLF2 was carried out through determination of its primary structure using the PROTEAN software of the DNASTar based on its flexibility, antigenic index, and surface probability.





confirms again the previous report (33). However, the future study needs to be carried out to determine whether BGLF2 possess similar functions with its homologues. It is reported that almost every protein is labeled with a correct subcellular localization signal, and various subcellular localizations of a specific viral protein may play diverse roles in the course of infection (34). Since BGLF2 is a capsid binding protein, in the future works, of course, it is crucial to identify the functions of the potential subcellular localization signal (including nuclear localization signal and nuclear export signal) of the BGLF2 in the process to virus replication, especially in the encapsidation process or viral assembly.

Protein phosphorylation is one of the most normal and essential types of protein modifications and certain aspects of the cell process, for instance, signal transduction, proliferation, differentiation, and metabolism, all of which are prone to be regulated by the activities of certain protein kinases and protein phosphatases upon pivotal target proteins. Applying phosphorylation site prediction we have shown that there are 15 potential phosphorylation sites in the EBV BGLF2, including 6 serine, 5 tyrosine, and 4 threonine residues. It is well known that tyrosine phosphorylation is associated with the modification of the protein translocation from the cytoplasm to the nucleus during the course of productive viral infection (35). Phosphorylation is also implicated in the replication of many herpesviruses (36). Indeed, modification by the phosphorylation has been described for several herpesvirus tegument proteins such as the process of tegument dissociation from the incoming nucleocapsids likely occurs as a result of phosphorylation by cellular and viral kinases (32). Thus, phosphorylation of EBV BGLF2 may also play an important role during EBV infection, perhaps in modulating its proper localization (37, 38), stable capsid structure assembling (39), or playing a potential regulatory role in the affinity for DNA (40), as well as virion morphogenesis, and egress (32).

In addition, sequence analysis indicated no potential transmembrane domain and signal peptide in EBV BGLF2, but it contained three N-linked glycosylation sites (Asn-X-Ser/Thr). In conformity with this prediction, pseudorabies virus UL16 was predicted to contain one potential N-linked glycosylation signal, but N-glycosidase F treatment experiment demonstrated that it is not N-glycosylated (41). Therefore, more works are required to establish whether BGLF2 is N-glycosylated.

The hydrophobicity and hydrophilicity analyses demonstrated that in BGLF2 the proportion of hydrophobic region was larger than the hydrophilic region, which might represent the internal and surface

regions of the protein, respectively. Furthermore, the B-cell epitopes of BGLF2 were predicted using DNASTar PROTEAN programs based on its flexibility, antigenic index, and surface probability by the determination of its primary structure (42-44). The results indicated that the improved knowledge of the antigenic and structural features of the BGLF2 resulting from this work might generate suitable approaches for developing new antibodies and immunoassays for the application in the clinical diagnosis of EBV. Besides, the secondary structure prediction revealed that BGLF2 contained 7 potential  $\alpha$ -helix regions and several possible random Coil and  $\beta$ -strand. Moreover, its 3D conformation also found to consist of the 7 potential  $\alpha$ -helix and two potential  $\beta$ -strands (linked by  $\beta$ -turn) and a number of hydrogen bonds. Therefore, we speculated the component of these structures of BGLF2 might be engaged in its self-association or the interactions with other components of the nucleocapsid (45-47), tegument (46-48), envelope (29), or the host proteins (49).

#### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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#### Supplementary Files

This manuscript includes supplementary file accessible on <http://www.ijbiotech.com>.

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