Epstein-Barr virus Associated B-cell lymphomas: Pathogenesis and Clinical Outcomes

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Abstract

Epstein-Barr virus (EBV) is a ubiquitous human γ-herpesvirus that establishes a life-long asymptomatic infection in immunocompetent hosts. It is also found to be frequently associated with a broad spectrum of B-cell lymphomas predominantly seen in immunodeficient patients. In spite of many resemblances, these EBV-linked lymphoproliferative disorders display heterogeneity at the clinical as well as the molecular level. Moreover, EBV-associated lymphoproliferative diseases differ in their differential expression patterns of the EBV-encoded latent antigens which are directly related to their interactions with the host. EBV-driven primary B-cell immortalization is linked to the cooperative functions of these latent proteins that are critical for perturbing many important cell-signaling pathways maintaining B-cell proliferation. Additionally, it is used as a surrogate model to explore the underlying mechanisms involved in the development of B-cell neoplasms. Recent discoveries have revealed that a number of sophisticated mechanisms exploited by EBV during cancer progression. This will be instrumental in the design of novel approaches for therapeutic interventions against EBV associated B-cell lymphomas. This review limits the discussion to the biology and pathogenesis of EBV associated B-cell lymphomas and the related clinical implications.

Background

Epstein-Barr virus (EBV), a member of γ-herpesvirus family, is the first human tumor virus that was originally identified in cultured lymphoblasts from Burkitt's lymphoma (BL) by Epstein and Barr in 1964 (1). Subsequent studies demonstrated that it is the causative agent of infectious mononucleosis (IM) and >90% of the worldwide population is asymptomatically infected by EBV (2, 3). EBV infects both B-lymphocytes and epithelium cells and intermittent reactivation and virus replication in the oropharyngeal epithelia allow the spreading of EBV infection and latent infection in B-lymphocytes (2, 3).

During latent infection, to maintain the viral genome and to successfully evade the host-cell immune surveillance, EBV express a small subset of genes, including six nuclear antigens (EBNA-1, -2, -3A, -3B, -3C, and -LP), three latent membrane proteins (LMP-1, -2A, and -2B), two small noncoding RNAs (EBER-1 and 2), and BamHI-A rightward transcripts

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(BARTs) (2–4). The differential expression pattern of these latent genes defines the distinct latency programs associated with the types of cancers (4). Type III latency, also referred to as the 'growth program', expresses all the latent antigens and is typically found in EBV associated post-transplant lymphoproliferative diseases (PTLDs), AIDS associated lymphomas and lymphoblastoid cell lines (LCLs) (4). *In vitro*, EBV can infect primary B-lymphocytes to generate continuously proliferating LCLs, in which four of the viral latent antigens – EBNA-2, -3A, -3C and LMP-1 are shown to be indispensable (4). Type II latency is characterized by a more restricted latent gene expression pattern (EBNA-1, LMP-1, -2), and is associated with Hodgkin's lymphoma (HL) and nasopharyngeal carcinoma (NPC). Type I latency predominantly expresses EBNA1 and is associated with Burkitt's lymphoma (BL) and gastric carcinoma (GC) (2, 5, 6).

One of the most significant features of EBV infection is the engagement of different cell-types and associated diseases (4). EBV is shown to be associated with a wide spectrum of human cancers including both hematopoietic and epithelial tumors most preferentially in post-transplant and AIDS patients (7). Despite its documented infection in T-lymphocytes and epithelial cells, EBV has a major preference for B-cells and under certain circumstances the infected carrier B-cells can transform into malignant B-cell lymphomas (4). Due to the preferential infection in B-cells, B-cell lymphomas are most prevalent among other EBV-associated lymphoproliferative disorders, which include HL, BL, PTLDs, lymphomatoid granulomatosis, senile EBV-associated B-cell lymphoproliferative disorders and many AIDS-associated B-cell lymphomas (4, 7). Given the escalating list of EBV-associated human cancers, the World Health Organization (WHO) has classified EBV as a 'carcinogenic agent' in 1997 (8).

Due to the critical association of EBV-associated lymphoproliferative diseases with viral latency, the functions of the latent antigens have been extensively studied. Nevertheless, further understanding the EBV life cycle, its genetic regulation, and the underlying molecular mechanisms are essential to fully comprehend the pathophysiology of EBV infection in the spectrum of EBV-linked lymphoproliferative syndromes and development of novel therapies. Current knowledge has allowed us to infer that EBV-encoded latent antigens have evolved many sophisticated strategies for manipulating important cellular pathways in the development of several human cancers (5). The important steps involved in EBV mediated B-cell transformation by the latent antigens are illustrated in Figure 1 and the biological functions of the latent proteins are discussed in detail hereunder and summarized in Table 1.

Contribution of latent antigens to B-cell transformation

EBNA-1 transcripts initiate from four different promoters – Wp, Cp, Qp and Fp (2). EBNA-1 is transcribed from Wp early during primary infection and switched over to Cp after establishing the host cell-transformation (2). During type I and II latency, Cp and Wp are epigenetically silenced and EBNA-1 transcription is initiated from Qp, which is regulated in a cell-cycle dependent manner (2). Interestingly, Fp is activated during the lytic cycle (2). EBNA-1 is essential for replication and stable persistence of episomes in EBV-infected proliferating cells and it is the only EBV encoded antigen that is consistently

expressed in all EBV-associated tumors (9). EBNA-1 contains two major functional domains - a C-terminal viral DNA binding domain at OriP, and an N-terminal host-chromosome tethering domain. An X-ray crystallographic 3D structure of EBNA-1 reveals structural similarities with the functional homolog of human papillomavirus (HPV) encoded E2 protein (10, 11). Moreover, protein 3D structure prediction software also indicated similar structural folds to another functional homolog, the Kaposi's sarcoma associated Herpesvirus (KSHV) encoded LANA protein (10, 11). This strongly suggests that EBNA-1 may serve as an attractive therapeutic target for inhibition of viral latent replication and persistence in EBV associated tumors (11). In addition, increasing evidence suggests an oncogenic role for EBNA-1 in the progression of EBV-associated human cancers. For example, down-regulation of EBNA-1 expression using RNA interference in many EBV-positive cells decreases cell proliferation and survival (12). In agreement with these observations, over-expression of a dominant-negative EBNA-1 mutant increases cell death in EBV-positive BL cells, suggesting an anti-apoptotic role for EBNA-1 (12). Furthermore, EBNA-1 expression increases metastasis in nude mice through blocking the activity of metastasis suppressor protein, Nm23-H1 (13). In addition, mice carrying an EBNA-1 trans-gene under control of the Ig heavy chain enhancer develop lymphomas (9). Furthermore, EBNA-1 may modulate its anti-apoptotic function by interacting with the host deubiquitinating enzyme HAUSP (14). In response to DNA damage, HAUSP interacts with and stabilizes p53. However, in EBV infected cells EBNA-1 can efficiently block the interaction between HAUSP and p53 resulting in ubiquitin-proteasome mediated degradation of p53 (5, 14). It should be noted that the effects of EBNA-1 on apoptosis are not only restricted to the regulation of p53 expression levels but also disrupts PML nuclear bodies in NPC cells (15), and leads to genomic instability by activating the recombinase-activating genes RAG-1 and RAG-2 (9). Although, it has long been thought that EBNA-1 expressing cells escape from cell-mediated immune system by blocking the MHC class I-restricted presentation, recent studies show that EBNA-1 can be presented to both CD4 and CD8-positive T cells. This allows EBNA-1 to be used as a potential target for immunotherapy against EBV-associated tumors (2).

EBNA-2 plays a critical role during EBV mediated host cell immortalization by coordinating the transcriptional level of viral as well as several cellular genes (3, 16). Together with EBNA-LP, EBNA-2 is the first EBV latent antigens detected after primary B-cell infection and suggested to be involved in G0 to G1 phase transition (2). Since EBNA-2 does not bind DNA directly, the regulation of its transcriptional activity largely depends on the interaction with RBP-Jκ (CBF1), a major Notch signaling mediator (2). EBNA-2 transactivates cellular genes, which include the B-cell activation markers CD21, and CD23 and c-myc, hes-1, and runx3 (16). Among viral genes which are activated by EBNA-2 include LMP1, LMP2 and the Cp promoter which regulates transcription of EBNA genes (17). Two serologically distinct EBNA-2 proteins have been indentified based on significant sequence diversity in EBV types 1 and 2, which also directly relates to functional consequences such as B-cell transformation *in vitro* (3). Although EBNA-2 is essential for initial growth transformation of EBV-infected B-cells *in vitro*, its expression may be less critical during cancer propagation as seen in most cases of EBV-linked tumors, apart from those in immuno-suppressed patients (2).

EBNA-LP (also known as EBNA-5) is concurrently expressed with EBNA-2 in infected naïve B-cells (3). Although a number of studies have suggested that EBNA-LP is important in B-cell growth transformation through co-activation of EBNA-2 mediated transcriptional activity of the viral oncoprotein LMP1, the precise mechanistic details of its contribution remains elusive (2, 3). EBNA-LP is an atypical viral protein comprised of a 22- and 44-amino-acid variable repeat region derived from two exons (W1 and W2) found in the viral internal repeated region 1 (IR1) and a unique 45-residue C-terminus derived from the Y1 and Y2 exons, which gives rise to multiple protein species ranging from 20–130 kDa during initial infection due to the intrinsic number of repeats in the specific viral genome in coordination with alternative splicing (2, 3). However *in vitro* transformed LCLs express few isoforms, and interestingly its overall expression is quite restricted in most of the EBV associated tumors (2). Genetic studies revealed the importance of the C-terminal 45-residues as a deletion of this particular domain resulted in viruses that immortalized B-cells with less efficiency (2). Although EBNA-LP appears to be dispensable for growth transformation, recent biochemical studies have suggested that it may contribute to this process by critically regulating several important host components activity. EBNA-LP was shown to form complexes with major tumor suppressor proteins p53 and pRb as well as cellular PKCs such as HA95 and HAX1, thereby facilitating the G0 to G1 phase transition of resting primary B-cells after EBV infection (2, 18).

The EBNA3 genes (-3A, 3B and -3C) are in tandem sequence in the EBV genome and are transcribed as alternately spliced transcripts from the Cp promoter (19). These nuclear proteins share approximately 30% sequence homology, and are assumed to be derived from gene duplication events (2, 20). These proteins share more than 70% sequence homology between EBV type 1 and type 2 strains, mainly due to variations in the C-terminal repeat regions, and thus allowing strain typing (2). Reverse genetics have shown that EBNA-3B is dispensable, whereas -3A and -3C are absolutely necessary for B-cell growth transformation along with EBNA-2 and LMP-1 (19). However, all three proteins affect global transcriptional activities including viral and several cellular gene expression patterns during host cell immortalization by modulating the EBNA2/RBP-Jκ driven transactivation process (19, 20). EBNA-3A together with EBNA-3C facilitates LCL outgrowth through epigenetic repression of the pro-apoptotic protein Bim and the cell cycle inhibitor p16^{INK4A} gene expression (19). EBNA-3C up-regulates cellular CD21 and c-myc expression and augments EBNA2-driven up-regulation of LMP1 expression and down-regulates Cp-promoter activity (2, 21). In addition to their role in transcriptional regulation, EBNA-3A and -3C also play an important role in cell-cycle regulation. EBNA-3C resembles Adenovirus E1A and HPV E7 proteins as its expression efficiently overrides various cell-cycle checkpoints in response to genotoxic stress through blocking p53-dependent apoptotic activity (22, 23) as well as by escalating ubiquitin-proteasome mediated degradation of pRb and p27^{KIP1} proteins (24–26). Besides, both EBNA-3A and -3C co-operate with oncogenic H-ras in transforming primary rat embryonic fibroblasts (REFs) (5, 27). Studies from our lab have demonstrated that EBNA-3C interacts with a wide range of cellular proteins involved in cell-cycle, apoptosis and epigenetic-transcriptional regulations, such as Cyclin A, Cyclin D1, SCF^{Skp2}, pRb, c-Myc, p53, ING4, ING5, Mdm2, p300 and HDAC1, which contributes to the B-cell transformation (5, 6, 21, 22, 28, 29). Moreover, our group has shown that both EBNA-1 and

-3C interact with the metastasis suppressor protein Nm23-H1 and positively modulates its NDP kinase and histidine kinase activities (5, 13). Furthermore, EBNA-3C together with Nm23-H1 blocks Necdin-mediated growth suppression and anti-angiogenic activities, suggesting their critical role in cancer metastasis and these antigens thus could serve as potential candidates for prophylactic vaccination strategies (5).

LMP1 is encoded by three exons and is an integral membrane protein with six hydrophobic membrane-spanning segments and a C-terminal cytoplasmic section (2). Possibly through associating with the intermediate filament protein vimentin, LMP-1 forms patches in the plasma membrane (2). Subsequent mutational analyses showed that the conserved N-terminal and the first two trans-membrane segments of LMP-1 are responsible for membrane aggregation essential for B-cell immortalization (2). LMP-1 expression has been shown to have growth transforming potential in rodent fibroblasts as it promotes cellular alterations that are usually coupled with primary infection and transformation of B-cells. Moreover, in B-cells LMP-1 expression blocks p53-mediated apoptosis via transactivation of the anti-apoptotic factors bcl-2 and A20 (30). Interestingly, in agreement with this observation, these genes are also shown to be up-regulated in LMP-1 transgenic mice (31). In addition, LMP-1 up-regulates IL-10 expression that stimulates B-cell proliferation as well as reduces immune response (32). Most importantly, LMP-1 augments host cell growth by mimicking CD40 mediated NF-κB signaling pathway via interaction with tumor necrosis factor receptors (TNFR)-associated factors (TRAFs) and TNFR-associated death domain protein (TRADD) (33). LMP-1 interaction with TRAF-1 and -3 leads to elevated expression of many B-cell activation markers, including CD23, CD39, CD40, CD44, and HLA class II and the cellular adhesion components LFA-1 and ICAM-1 (33). Moreover, LMP-1 modulates JAK/STAT, ERK MAPK, IRF and Wnt signaling pathways (5, 33). These cell signaling activities are mediated by the three cytoplasmic C-terminal activating regions (CTAR-1, -2 and -3) of LMP-1 (2, 33). LMP-1 also can affect the biogenesis of both cytokine and cytokine receptor, which further alters overall angiogenesis and inflammatory responses, thereby contributing to immune escape and cancer propagation (2, 3).

LMP-2 gene encodes 2 isoforms of the hydrophobic integral membrane protein – LMP-2A and -2B that are transcribed from two distinct promoters after circularization of the EBV genome (2, 3). Similar to LMP-1, they are comprised of trans-membrane domains and a hydrophilic C-terminal domain (2, 3). In comparison to LMP-2B, LMP-2A contains an extra 118-residue cytoplasmic N-terminal domain encoded in exon 1, which interacts with the cellular tyrosine kinases Lyn and Syk to block B-cell receptor (BCR) signaling, and also prevents lytic-cycle induction (34). Although LMP-2 is not required for B-cell transformation, studies have clearly suggested that it is essential for long-term persistence for the viral episome by providing temporal growth and B-cells survival signals in the lymphoid organs (3). LMP-2A appears to play a central role in the persistent infection of B-cells. Recent findings suggest that LMP-2B modulates LMP-2A function on regulating BCR signaling and thus driving the latently infected B-cells to lytic re-activation (2, 3).

Among two EBV non-polyadenylated RNAs (EBERs), EBER-1 is abundantly expressed, although there is instances when EBER-2 is expressed at elevated level (2). They interact with cellular autoantigen La and the ribosomal protein L22 (35). In addition, they block

apoptosis, suppress anti-viral effects of interferon (IFN)-α and -γ and induce IL-10 production, perhaps mediated by binding to and blocking the function of the IFN-induced double-stranded RNA-activated protein kinase PKR (2). Recently, EBERs have been shown to bind RIG-I and activate its downstream signaling, which further activates type-I IFNs (36). Moreover, EBERs can induce IL-10 via NF-κB-independent IRF3 activation in BL cells (37). Although it was initially suggested that EBERs are not essential for EBV-mediated cell growth transformation, they may actively participate in cancer progression via modulation of the host-immune signaling activities (37).

EBV was the first human virus shown to encode microRNAs that mapped to the lytic BHRF1 and latent BART regions of the genome (3). BARTs are transcribed from Bam H1A region of the viral episome (3). Although these transcripts can be detected in various EBV-associated tumors, they are expressed at elevated levels in NPCs (3). Several open reading frames (ORFs) have been identified within the differently spliced BARTs (3). The function of most of the BARTs is still under investigation but their detection in infected B-cells and in many established EBV-associated lymphomas suggests that they might have important role in viral persistence and pathogenesis.

Clinical-Translational Advances

In spite of the current molecular advancements in exploring the underlying mechanisms in EBV latent infection associated with the development of its related diseases, the optimal management in controlling many EBV-associated B-cell lymphomas remains largely inadequate. However, recent studies have suggested that combined therapeutic approaches of specific antiviral compounds targeting specific viral antigens with either T-cell based immunotherapy or targeted monoclonal antibodies show promising outcomes against EBV-associated tumors.

In order to stimulate the host immune responses as a therapeutic strategy against EBV-associated B-cell lymphomas, efforts have been made in patients who have developed PTLD after allogeneic hematopoietic stem cell transplantation (HSCT) (3, 38). Adoptive immunotherapy using EBV-specific cytotoxic T-lymphocytes (CTLs) has also been shown to be particularly useful in this context. The CTLs can be generated from a healthy donor and infused directly into the recipient patient to reinstate immuno-competence (3). The infusion of un-manipulated donor cells resulted in high response rates, although there is a considerable risk of graft-versus-host disease (GVHD) due to alloreactive-CTLs (3). These aforementioned B-cell related lymphomas represent an attractive target for CTL based immunotherapy as the transformed B-cells express the full array of latent antigens. Moreover, the potential for success using this approach in clinical trial is facilitated by the relative ease to obtain EBV-specific CTLs in large numbers using *in vitro* transformed EBV positive LCLs (3). In patients with solid organ transplants (SOT) who develop EBV-associated lymphoproliferative syndromes, EBV-specific CTLs have shown restricted response as the tumor develops in recipient B-cells (39). SOT patients require continuous immuno-suppression to prevent graft rejection. Although adoptive transfer of EBV-specific CTLs may help in restoring immuno-competence, CTL function is still partially impaired by the immuno-suppressive drugs. Currently strategies to genetically modify EBV-specific

CTLs for resistant against immuno-suppressive drugs are been developed (40). Generation of EBV-specific CTL in this patient population offers various challenges compared with HSCT recipients, as the SOT donor is not HLA-matched and usually deceased (40). Several groups have successfully administered autologous EBV-specific CTLs as prophylaxis in SOT recipients (40, 41). However, making autologous CTLs are sometimes not feasible. Consequently, a panel of CTLs is typically generated from healthy donors and only the HLA-matched CTLs are used for the treatment (42). In contrast, there are limited clinical studies that are documented with EBV-CTLs for other EBV-linked lymphomas, and are mostly related to HL with a partial response due to alloreactive-CTLs (43). In addition, to protect against initial infection or to enhance adoptive immunity in patients with EBV-associated neoplasm the strategies would be to develop vaccines using combinations of several defined EBV epitopes or linearly joined epitopes expressed as chimeric polypeptides to stimulate EBV-specific CTL immunity (44).

To date, several antiviral agents have shown encouraging anti-EBV activity. However, most of these drugs display broad-spectrum activity against other viruses and show limited efficacy against EBV-latent infection (2). For instance, drugs such as acyclovir and ganciclovir are nucleoside analogues that efficiently target the viral thymidine kinase (TK) important for EBV lytic replication (45). In contrast, in latent EBV-associated B-cell lymphomas where TK is not expressed anti-viral therapy would be ineffective (45). To overcome this obstacle, ganciclovir is administered together with arginine butyrate, which induces lytic cycle and TK production and have shown positive results in patients with PTLD (38). Similarly, other chemotherapy agents may also induce lytic infection, and one study has shown that the combination of gemcitabine/doxorubicin with ganciclovir is highly effective for the inhibition of EBV-driven lymphoproliferation in SCID mice (46). The viral DNA polymerase is another target for other antiviral compounds such as foscarnet and cidofovir, which show remarkable anti-tumor effects in patients (47). Interestingly, Rituximab, a monoclonal antibody against CD20 either alone or coupled with cidofovir eradicates PTLD symptoms in organ-transplant patients with a success rate of more than 60% (48, 49). Moreover, a HDAC inhibitor azelaic bishydroxamic acid, and a retroviral agent zidovudine have been used effectively against EBV transformed B-lymphocytes from AIDS patients (50, 51). Several other virus-targeted therapeutic approaches have been currently under consideration. One such strategy is based on the induction of EBV lytic-cycle (45). A better understanding of the viral and cellular factors that critically modulate lytic-cycle mode will allow us to evaluate the clinical potential for such a strategy. Moreover, therapeutic advancements based on drugs such as hydroxyurea capable of eliminating the viral episome from tumor cells may have clinical significance (38). Strategies undertaken with RNA interference (RNAi) have been largely accepted for the treatment of many virus mediated cancers, where RNAi designed against viral latent antigens crucial for transformation, such as LMP1, have anti-tumor effects without altering the physiology of the cellular networks (38, 52, 53). Recently, we have also shown that a knockdown strategy for EBNA3C gene efficiently blocks LCL growth as well as induces apoptosis, leading to the enhancement of the therapeutic window against B-cell lymphoma (29). In addition, a number of eccentric antiviral agents including, plant flavanoids have also shown a variable degree of anti-EBV activity (54). Thus, the future of therapeutic

developments against EBV-associated B-cell lymphomas shows a great deal of promise which should translate in enhancing patient care.

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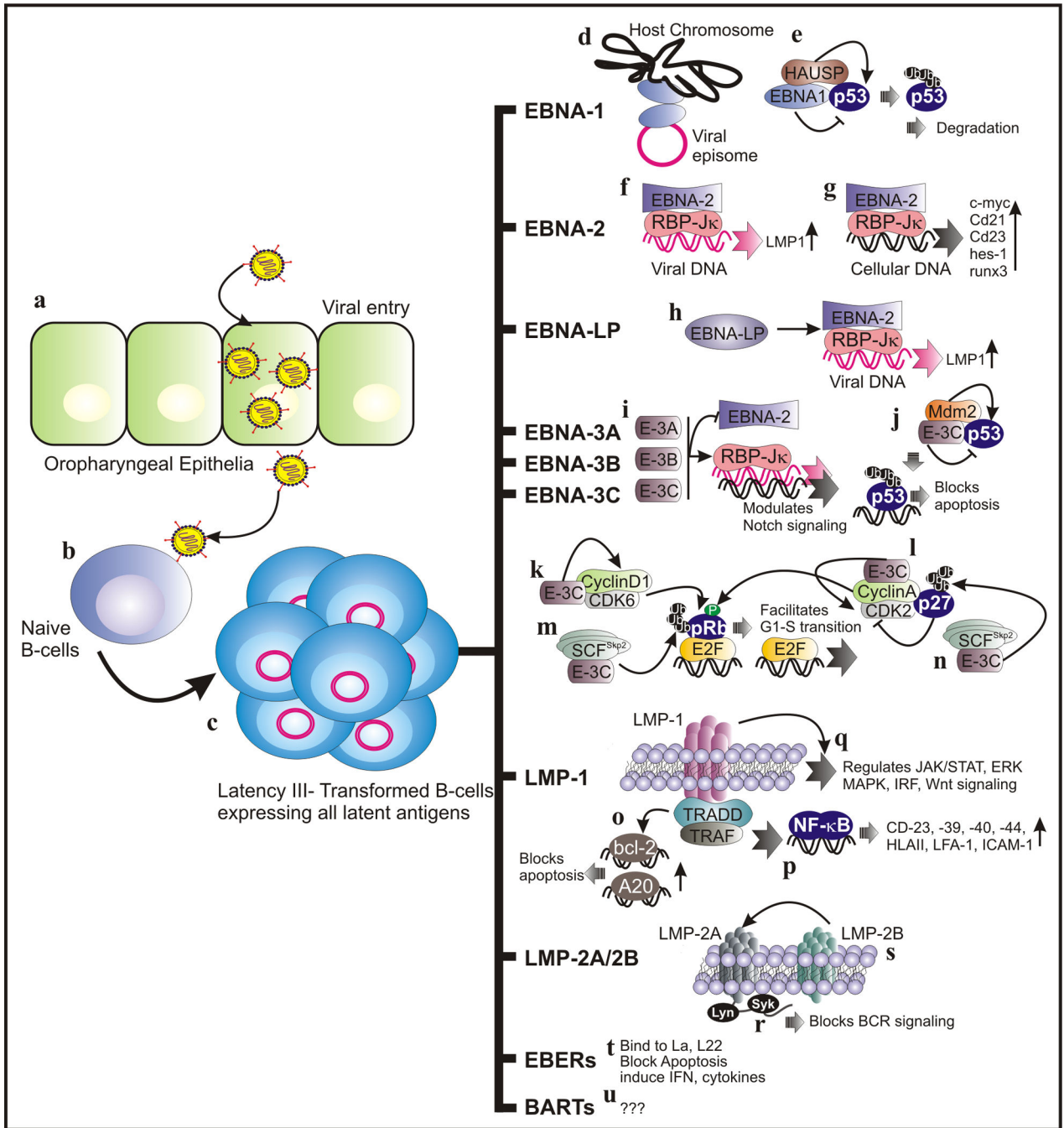
Figure 1. The salient features of EBV latent antigens in B-cell lymphomas

After initial infection of oropharyngeal epithelial cells (**a**) EBV primarily infects naïve B-cells (**b**) and subsequently the infected B-cells are growth transformed (latency III), expressing the full repertoire of latent antigens (**c**). EBNA-1 tethers viral episome to host chromosome and assists viral replication and segregation (**d**). EBNA-1 blocks the interaction between p53 and HAUSP and induces its degradation (**e**). EBNA-2 regulates a wide range of both viral (**f**) and cellular (**g**) gene transcription via RBP-Jκ. EBNA-LP promotes EBNA-2 mediated gene transcription (**h**). EBNA-3 proteins (-3A, -3B and -3C) by

blocking EBNA-2 association with RBP-Jκ modulate viral gene transcription as well as Notch signaling pathway **(i)**. EBNA-3C forms a ternary complex with p53 and Mdm2, recruits the Mdm2 E3 ligase activity to ease its degradation and blocks p53-dependent apoptosis **(j)**. EBNA-3C binds to and enhances the kinase activity of both Cyclin D1/CDK6 **(k)** and Cyclin A/CDK2 **(l)** complexes to phosphorylate pRb. In addition, EBNA-3C recruits SCF^{Skp2} E3 ligase activity on hyperphophorylated pRb **(m)** and p27^{KIP1} **(n)** for degradation, thereby facilitating the transition of G1 to S phase. LMP-1 inhibits p53-mediated apoptosis by transactivating bcl-2 and A20 genes **(o)**. LMP-1 augments NF-κB signaling to transactivate numerous cellular genes via interaction with tumor necrosis factor receptors (TNFR)-associated factors (TRAFs) and TNFR-associated death domain protein (TRADD) **(p)**. LMP-1 also modulates JAK/STAT, ERK MAPK, IRF and Wnt signaling pathways **(q)**. LMP-2A blocks B-cell receptor (BCR) signaling through interaction with cellular tyrosine kinases Lyn and Syk **(r)**. LMP-2B regulates LMP-2A function **(s)**. EBERs block apoptosis, bind to La and L22 cellular proteins and induce IL-10 cytokine and type I IFN **(t)**. The biological function of BARTs is under investigation as to their role in development of B-cell lymphoma **(u)**.

Table 1

Proposed functions of EBV latent antigens and the associated B-cell lymphomas.

Latent proteins	Functions	Associated Lymphomas	Latency type
EBNA-1	Essential for B-cell immortalization, replicates EBV genome, segregates viral episomes at mitosis, blocks interaction between HAUSP and p53 to facilitate p53's degradation.	Burkitt's lymphoma Hodgkin's disease AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	I, II, III
EBNA-2	Transcriptional co-activator that up-regulates expression of viral (LMP1) and cellular genes (c-*myc*), essential for B-cell immortalization	AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	III
EBNA-3A	Essential for B-cell immortalization of cell, interacts with RBP-Jκ, regulates Notch-signaling pathway.	AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	III
EBNA-3B	Not essential for B-cell immortalization, interacts with RBP-Jκ.	AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	III
EBNA-3C	Essential for B-cell immortalization, overcomes various checkpoints in cell-cycle, interacts with RBP-Jκ, activates LMP1, blocks p53-dependent apoptosis, enhances kinase activity of both Cyclin D1/CDK6 and Cyclin A/CDK2 complexes, induces degradation $p27^{KIP1}$ and pRb, stabilizes c-Myc, Cyclin D1 and Mdm2, manipulates host chromatin remodeling machinery.	AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	III
EBNA-LP	Interacts with EBNA2 to inactivate p53 and pRb, interacts with transcription factors in notch signaling pathway, contributes to B-cell immortalization.	AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	III
LMP-1	Mimics CD40 ligand binding signal, stimulates bcl-2 and a20 expression to block apoptosis, acts a constitutively active receptor for stimulating many cellular genes, regulates NF-κB, JAK/STAT, ERK MAPK, IRF and Wnt signaling pathways, essential for B-cell immortalization.	Hodgkin's disease AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	II, III
LMP-2A and -2B	Drives EBV into latency, LMP-2A blocks BCR signaling and -2B assists its function, not essential for B-cell immortalization.	Hodgkin's disease AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	II, III
EBERs	Form complexes with La and L22, associate with PKR, induce IFN and IL-10, bind to RIG-1 to activate type I IFNs, not essential for B-cell immortalization.	Burkitt's lymphoma Hodgkin's disease AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	I, II, III
BARFs	Protein products may modify Notch signaling, not essential for B-cell immortalization	Burkitt's lymphoma Hodgkin's disease AIDS-associated lymphomas Posttransplant lymphoproliferative disorders	I, II, III