HHS Public Access

Author manuscript

Curr Opin Virol. Author manuscript; available in PMC 2018 March 26.

Published in final edited form as:

Curr Opin Virol. 2015 October ; 14: 138–144. doi:10.1016/j.coviro.2015.09.007.

Epstein-Barr Virus Latency: Current and Future Perspectives

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Abstract

EBV drives resting B cells to continuous proliferating latently infected cells. A restricted program of viral transcription contributes to latency and cell proliferation important for growth transformation. Recent interest in latency and transformation has provided new data about the roles of the EBV encoded latent proteins and non-coding RNAs. We broadly describe the transcription, epigenetic, signaling and super-enhancer functions of the latent nuclear antigens in regulating cellular transcription; the role of LMP2 in utilization of the autophagosome to control cell death, and the association between LMP1, the linear ubiquitin chain assembly complex and TRAF1 which are important for transformation. This review explores recent discoveries with new insights into therapeutic avenues for EBV related malignancies.

Introduction

Epstein-Barr virus (EBV) infection is causally linked to several malignancies including endemic Burkitt's lymphoma, Hodgkin's lymphoma, post-transplant lymphoproliferative disorders, nasopharyngeal carcinomas, gastric carcinoma and leiomyosarcoma (36). Based on seroprevalence, 95% of human adults carry EBV world-wide for their life-time (20). Primary EBV infection causes infectious mononucleosis in at least 125,000 cases in the United States every year and the incidence of EBV associated malignancies is estimated to be 200,000 new cases each year world-wide. The strong bias of the incidence of specific tumor entities in different parts of the world strongly suggests, that environmental factors including co-infections, social status, host genetics, immune status of the infected host and different EBV strains all impact on pathogenesis (9, 18, 36). Neither EBV vaccines nor EBV specific anti-viral drugs have been approved for patient treatment.

EBV is a γ-herpesvirus that primarily infects human epithelial and B cells and establishes a lifelong persistent and asymptomatic infection in memory B cells which circulate in the peripheral blood (47). Similar to other herpesviruses, the EBV life cycle can switch between

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latent and lytic state. Occasional reactivation from latency and virus production is triggered by environmental stimuli but tightly controlled by the immune system of healthy individuals (20, 47).

EBV infects human primary B cells, activates their cell cycle during early infection and induces the permanent proliferation of the infected lines *in vitro* after a programmed expression pattern that uniquely allows for transition from a resting to a full blown transformed lymphoblastoid cell line (15, 39). Growth transformation of B cells by EBV *in vitro* is based on the concerted action of Epstein-Barr virus nuclear antigens (EBNAs) and Latent membrane proteins (LMPs) (39, 47). A unique and characteristic feature of EBV is its capability to establish distinct latent gene expression patterns in vivo and in cultured cells (named type 0, I, II and III) in resting and proliferating cells (47). EBV growth transformed B cells express a viral transcriptional program, referred to as latency III, which is characterized by the co-expression of EBNA2 and EBNA-LP with EBNA-1, EBNA-3A, -3B and -3C, as well as LMP1, LMP2A and LMP2B (20, 39).

Importantly, the different latent expression programs are found in lymphoproliferative and epithelial malignant diseases, as most recently reviewed in (34). Distinct viral gene expression programs, including coding and non-coding RNAs control cellular gene expression, signal transduction, proliferation and survival of infected cells. Using the cellular replication machinery, the latent viral genome is maintained in proliferating cells as a replicating nuclear episome with expression of the viral encoded latent antigen EBNA1, and segregates to daughter cells during mitosis (1, 12). Both, the viral and cellular gene expression programs are tightly controlled by cellular signaling activities, and epigenetic mechanisms, including DNA methylation, chromatin modification and nucleosome occupancy. We will focus on these major processes and their relevance in terms of latency infection and proliferation.

Public resources for EBV research

Cost-efficient high throughput sequencing techniques have fostered many aspects of EBV research during the last years. The ENCODE project lists a wealth of data on primary human B cells and 42 EBV immortalized B cell lines (named GM followed by a five figure number) which have been analyzed for multiple parameters by the ENCODE consortium and the results have been mapped to the GRCh37/hg19 human genome assembly (4). These data sets include RNA sequencing results, transcription factor binding sites, binding sites for architectural proteins, chromatin modifications, methylation status of the DNA, and the definition of functional genomic elements like enhancers and are continuously expanded. These cell lines have all been generated by infecting primary human B cells with the laboratory type 1 EBV strain (B95-8). Thus, all data sets describe the cellular epigenomes of Type III Latency infected B cells and are a growing and valuable resource for EBV research (10).

Although the first EBV sequence was published in 1984 (5), large scale sequencing of EBV genomes has been accomplished only recently (23, 31) and references therein]. Novel genome capture technologies similar to exome sequencing as well as bioinformatics tools

had to be developed. De novo sequencing and assembly of EBV genomes is still in its infancy but will likely provide new information in the near future. Approximately 100 genomes from malignant and non-malignant cells from different geographical origins have been sequenced. Based on the SNP variation and the frequency of non-synonymous nucleotide exchanges within the latent EBNA2 and EBNA3A, 3B and 3C genes, the genomes tend to cluster in two major groups that had been previously defined as the two major EBV types (4). In addition, intertypic recombinants can also be found. At present, the number of sequenced viral and cellular genomes is still insufficient to establish geographical links and correlate disease association with the specific viral variants to identify potential high risk strains. On a long-term basis this technological progress and the use of data sets provided by Hapmap should produce data sets sufficient to generate testable working hypotheses (18).

EBNA2, EBNA3A, EBNA3C preferentially bind to enhancers

Since EBNA2, EBNA-LP, EBNA3A, and 3C are co-expressed in type III latency, the association of these protein with cellular chromatin has been studied intensely by co-immunoprecipitation and high throughput sequencing. The sequencing results were mapped to reference genomes and the binding patterns were compared. Using published data sets these ChIP-Seq data as well as ChIP-PCR data were further analyzed for co-occurring chromatin signatures and transcription factor binding sites in type III latency at a genome wide level using data provided by the ENCODE consortium (16, 29, 33, 49, 50). The total number of genomic binding sites varied, ranging from approximately 5,000 to 20,000 significant peaks for individual viral factors in different studies. This discrepancy can be easily explained by the use of different biological reagents and bioinformatics parameters and tools. All studies agree that EBNA2 and the EBNA3 binding sites partially overlap and preferentially but not exclusively target enhancers. EBNA-LP preferentially occupies promoter sites consistent with a model by which EBNA2 and EBNA-LP establish chromatin loops of promoter and enhancer elements (33). EBNA3A and EBNA3C regulated promoter and enhancer regions were enriched for repressive polycomb chromatin signatures as exemplified by the selected EBNA3 target genes BCL2L11, the INK4b-ARF-INK4a locus and integrin receptor signaling genes (3, 28, 32, 471, 45). At the CXCL9/10 gene locus the polycomb signature is established by EBNA3A in a two-step process. EBNA3A competes with EBNA2 for binding to intergenic enhancer regions. Loss of EBNA2 causes enhancer inactivation and transcriptional silencing of the CXCL9 and 10 genes. The genes for CXCL9 and 10 reside in a co-regulated chromatin domain that is entirely marked by a polycomb signature in the silent EBNA3A positive state (16, 17). Transcriptional silencing of CXCL9 and 10 precedes the deposition of repressive marks by the polycomb complex. Polycomb is either directly but slowly recruited by EBNA3A. Alternatively, the enhancer region that is switched off could be polycomb modified by default. EBNA3C functions through a number of tumor suppressors and cell cycle regulators important in driving cells through the resting state to a growth transformed state by disruption of their normal activities (6, 22, 39, 40). Recruitment of E3 ligase for ubiquitination, and deubiquitination of target substrates to dysregulate their activities has shown the broad impact of EBNA3C in contributing to the transformed phenotype (37-38).

The assignment of enhancer and corresponding promoters in the cellular genome is hampered by the long distance relationships of both functional units when linear distances are looked at without considering the nuclear architecture in chromosome territories and topological association domains (TADs). TADs can be identified by high-resolution chromatin conformation capturing assays (29). TADs are mega-base size chromatin interaction domains and functional units, bordered by architectural proteins like CTCF, housekeeping and t-RNA genes that prevent the spread of heterochromatin (11). A quantitative study of EBNA2 binding sites identified a subpopulation of 888 highly active "EBNA2 super enhancers" that were characterized by high EBNA2 and B cell transcription factor density, elevated enhancer marks as H3K27 acetylation, and H3K4 mono-methylation levels (50). In parallel approximately 1,700 sites co-binding sites of EBNA2, EBNA-LP, EBNA3A and EBNA3C which also recruit NFKappaB, presumably activated by LMP signaling, were defined. Notably, EBNA2 super enhancers and approximately 86% of previously defined EBNA2 target genes co-localize in TADs (42, 50). EBNA2 was shown to establish long-distance physical interactions between enhancers and the promoter of one of its target gene c-myc (49, 50). Importantly, modulation of chromatin loops by EBNA3C correlates with repression although this would likely be only in the context of EBNA2 and other transcription activators (29).

In summary, EBNA2 exploits the epigenetic status and nuclear architecture of the B cell chromatin that is marked by the B cell specific transcription factor network to access cellular chromatin and regulate cellular transcription. EBNA3 proteins are engaged in transcription modulation through their interactions with a number of cellular regulatory processes including tumor suppressor activities, cell cycle regulation, as well as epigenetic changes that may be important for long range chromatin interactions. These interactions are likely to contribute globally to the transcription profile of EBV transformed latently infected B cells (See Figure 1).

Cellular adaptors which recruit EBNA2, EBNA3A and EBNA3C to chromatin

All enhancers bound by EBNA2 or EBNA3 proteins are co-occupied with B cell transcription factors. Whether EBNA2 directly interacts with any of these B cell transcription factors and uses these proteins as adaptor to the cellular chromatin has not been studied until recently. Both, EBNA2 and the EBNA3 proteins use cellular adaptor proteins to bind to cis-regulatory regions of its target genes which indirectly confer sequence specific DNA contact to the viral proteins (29). So far, the best studied cellular DNA adaptor protein of EBNA2 is the DNA binding protein CBF1/CSL, which directly binds to EBNA2. CBF1/CSL (CBF1 for C-promoter binding protein, Su(H) in *Drosophila melanogaster*, Lag1 in *Caenorhabditis elegans)* is also called RBP or RBPJκ. The second but less well characterized EBNA2 DNA adaptor is the PU.1 protein which is critical for activation of the viral LMP1 promoter by EBNA2. Like EBNA2 the EBNA3A, 3B and 3C proteins bind to CBF1/CSL but binding of these viral proteins is mutually exclusive and competitive (25). EBNA2 and CBF1/CSL chromatin binding sites widely overlap indicating that CBF1/CSL is an important DNA adaptor for EBNA2 (19). In contrast, only a minority of EBNA3A or EBNA3C binding sites co-localize with CBF1/CSL but enriched more strongly at BATF or at BATF/IRF4 and SPI/IRF4 binding sites, respectively. These findings might suggest that

EBNA3A and 3C can target chromatin independently of CBF1/CSL (19, 41). The recent finding that EBNA3C can bind to and stabilize IRF4 through degradation of its IRF8 partner is consistent with a concept that IRF4 recruits EBNA3C to chromatin (6). Furthermore, the induction of the cellular oncoprotein Pim-1 by EBNA3C results in increased p21 phosphorylation and its degradation mediated by the proteosomal degradation pathway (7). These studies are consistent with a role for EBNA3C in regulating a number of major cellular transcription and cell cycle regulatory factors for driving proliferation and survival of EBV infected cells. At EBV super enhancers, EBNA2, EBNA3A and 3C co-localized with CBF1 binding sites (41). The contribution of CBF1/CSL to EBNA3A and EBNA3C binding at these sites will require further exploration to fully understand the roles of these complexes. Early after infection, EBNA2 and EBNA-LP gene expression is followed by expression of the EBNA3 and LMPs which occurs by 48 hours after infection (2, 15). Interestingly, it has been reported that the maximal expression levels of EBNA3s and LMPs were not reached until approximately 21 days which is maintained in LCLs and needs further explanation (35). However, the timing of expression appears to be much earlier and may be a consequence of the percentage of cells that are initially infected, as well as the switch from the early lytic burst to a strict latent infection. Interestingly, EBV super enhancers and associated genes were strongly activated indicating that a delicate balance of activation and repression is established to maintain the transcription program in type III latency (See Figure 1).

The role of the latent membrane proteins in latency

The EBV encoded latent membrane proteins (LMPs) are primarily expressed in lymphoblastoid cell lines (LCLs) and Hodgkin's Lymphoma (HL) and nasopharyngeal carcinomas (NPCs) which are type III and type II latency, respectively (20, 39). In B-cells that are infected and transformed by EBV, LMP1 is well known to be the major viral oncoprotein and is demonstrated to be essential for the oncogenic process which drives B-cell transformation *in vitro* (13, 14, 48). Its primary functions are linked to activation of major cellular pathways including the NF-kB pathway, and the ERK, JNK and p38 signaling pathways (13). Deletions of LMP1 in genetic experiments resulted in failed transformation and loss of outgrowth of LCLs in culture. LMP1 expression also induces colony formation and expression of a range of cellular factors including CD40, ICAM1, CD21, LFA1 and other adhesion factors all contributing to establishment of latent infection and is essential for cell transformation (8, 13, 21). More recently, one study showed that a recombinant LMP1 deficient EBV was able to contribute to lymphomagenesis in vivo but required CD4 positive T cells suggesting that T cell may be contributing signals that can substitute for LMP1 including the activation of the TNF and CD40 signaling pathways (27).

LMP1 structure contains functional domains and motifs which includes 6 transmembrane domains and 2 regions in the carboxy terminal tail that are referred to as TES1 and TES2 (13). These two regions are critical regions in terms of their contribution for B cell transformation (See Figure 1) and constitutive signaling which essentially usurps CD40 functions (14). These activities have been carefully dissected over the last almost 2 decades at the molecular level in which LMP1 through its TES1 and TES2 domains can activate NF-

kB signaling through TRAFs leading to activation of p65/p50/p52 phosphorylated complex important for downstream gene transactivation (14).

The other latent membrane protein 2A (LMP2A) interestingly, can support LMP1 functions to some extent and contributes to growth transformation as well as suppression of B-cell receptor (BCR) signaling (26). LMP2A along with LMP2B also contributes to regulation of lytic reactivation of EBV (30). LMP2B actively impairs the activation of LMP2A when B cells are induced to reactivate by BCR cross-linking. Both forms of LMP2 are almost identical except for a 119 amino acid sequence in the amino terminal and has 12 transmembrane domains (26). It forms a phosphorylated membrane complex with the Src kinase family members Syk and Lyn for signal transduction (26). The inhibition of BCR activation by LMP2A can be challenged by inducing the PKC activities which results in enhanced cellular calcium levels (See Figure 1). Therefore the control of LMP2A BCR activation and calcium internalization by LMP2B effectively regulates EBV latency by actively blocking the switch from latent to lytic replication (30).

EBV encoded non-coding RNAs and their contribution to latent and persistent infection

EBV encodes a large number of small RNAs which is abundantly expressed in infected cells. The two most studies small noncoding RNAs are referred to as EBERs (46). Two major EBERs are highly expressed by EBV in all infected cells and are PolIII transcripts which are non-polyadenylated. These RNAs were deleted and showed no effective role in B cell transformation *in vitro* (20, 46). However, some contribution to proliferation by inducing growth of colonies *in vitro* and tumor induction *in vivo* demonstrates some of the potentially important roles of these 2 small viral encoded RNAs. Other roles of EBERs include the inhibition of PKR related phosphorylation of EIF2a which leads to inhibition of protein synthesis (43, 44). More recently studies have shown that the EBER2 transcript binds nascent RNA to regulate the transcription factor PAX5 binding to its site within the EBV terminal repeats (24). This clearly demonstrates a vital function of the EBERs in replication of the virus (See Figure 1). Furthermore, it also shows the contributions of cellular factors as well as the viral terminal repeats in this critical process which ensures the integrity of the viral DNA as replication proceeds.

The other small RNAs are predominantly from two major regions in the EBV genome, the BamH1, H fragment right reading frame 1 (BHRF1); and BamH1 A fragment right transcripts (BARTs) which encodes cumulatively over 25 miRNAs (20, 47). The EBERs and BART miRNAs are expressed in all infected cells, and described latency programs. However, the BHRF1 miRNAs are restricted and detected mostly in latency III program and early during infection when the genome is undergoing active transcription. It is likely that these latency programs exhibit a degree of plasticity as seen in other described programs referred to as latency IIa and IIb. However, it has become acceptable to acknowledge that there are always exceptions to the rule as it relates to EBV latency programs. A number of the targets have been validated and include a wide range of factors involved in transcription regulation, IFN induced chemokine CXCR3 and an NK ligand MICB (20), which is thought

to allow for a degree of stealth in terms of avoidance of the immune surveillance system (See Figure 1). Thus, these small RNAs probably provide a degree of survivability to the infected cells for long-term maintenance of the viral genome in the immune competent host.

Open questions and future perspectives

Based on availability of the biological material, the use of recombinant EBV strains and the wealth of information provided by public knowledge bases for EBV infected B cells, the control mechanisms defining latency III have been thoroughly studied and provided important insights into the function of individual viral factors. Future studies will need to quantify the equilibrium and transitional stages between the different types of transcription programs and further mechanistically define the contribution of B cell specified transcription factors in EBV controlled B cell enhancers. Comparable genome wide studies will need to be launched investigating the different states of latency in B cell malignancies, or epithelial host cells and their malignant counterparts. The contribution of the microRNAs and the EBERs continue to shed some light on the role of these viral encoded RNAs. How distinct are their roles and expression in the latency programs and how important are their individual contributions in terms of B cell transformation, epithelial cell infection and latent or lytic infection? The recent studies on super-enhancers provides important clues as to the cooperation of the latent nuclear antigens with cellular transcription factors. The mechanisms related to their activities and level of contributions in the context of the latent viral transcriptome is yet to be fully understood. Importantly, the regulatory functions of the LMPs working in consort with the EBNAs needs further exploration. One area that is still relatively unexplored is lytic infection in epithelial cells and the contribution to pathogenesis. It would not be a complete surprise if lytic antigens play a greater role contributing to progression of the multitude of EBV associated diseases. There is still so much more that is yet to be explored to fully comprehend the complexities related to EBV-driven malignancies.

Acknowledgments

This review was supported by public health funds from the National Institutes Health to ESR (P01CA174439, R01177423, R01171979, P30DK050306, P30CA016520, U54CA190158). ESR was also supported by the Avon foundation for women and is a scholar of the Leukemia and Lymphoma society of America. BK was supported by the Deutsche Krebshilfe and Deutsche Jose Carreras Leukamie-Stiftung (Grants 10-1963-Ke-1 and DJCLS R 07/11). We thank HCJ and EP for helping with the figure and critical reading of the manuscript.

References

1. Aiyar A, Aras S, Washington A, Singh G, Luftig RB. Epstein-Barr Nuclear Antigen 1 modulates replication of oriP-plasmids by impeding replication and transcription fork migration through the family of repeats. Virology journal. 2009; 6:29. [PubMed: 19265546]
2. Alfieri C, Birkenbach M, Kieff E. Early events in Epstein-Barr virus infection of human B lymphocytes. Virology. 1991; 181:595–608. [PubMed: 1849678]
3. Allday MJ. EBV finds a polycomb-mediated, epigenetic solution to the problem of oncogenic stress responses triggered by infection. Frontiers in genetics. 2013; 4:212. [PubMed: 24167519]
4**. Arvey A, Tempera I, Tsai K, Chen HS, Tikhmyanova N, Klichinsky M, Leslie C, Lieberman PM. An atlas of the Epstein-Barr virus transcriptome and epigenome reveals host-virus regulatory interactions. Cell host & microbe. 2012; 12:233–245. This comprehennsive study provides a plethora of information about the transcription and epigenome of EBV in the latently infected

cells. For teh first time a picture emerged which showed a clear pattern of regulation at the viral genome level and points to a more complex system for EBV gene expression. [PubMed: 22901543]

5. Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, Gibson TJ, Hatfull G, Hudson GS, Satchwell SC, Seguin C, et al. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature. 1984; 310:207–211. [PubMed: 6087149]

6**. Banerjee S, Lu J, Cai Q, Saha A, Jha HC, Dzeng RK, Robertson ES. The EBV Latent Antigen 3C Inhibits Apoptosis through Targeted Regulation of Interferon Regulatory Factors 4 and 8. PLoS pathogens. 2013; 9:e1003314. The identification of the IRF4 and 8 complex as important regulators of apoptosis and B cell development in EBV infected cells shows a critical link between the essential EBV transcription factor EBNA3C and its ability to stabilize IRF4 leading to downregulation of IRF8 by enhancing its proteasome-mediated degradation. [PubMed: 23658517]

7**. Banerjee S, Lu J, Cai Q, Sun Z, Jha HC, Robertson ES. EBNA3C augments Pim-1 mediated phosphorylation and degradation of p21 to promote B-cell proliferation. PLoS pathogens. 2014; 10:e1004304. The role of EBNA3C in regulating Pim-1 kinase leads to phosphorylation of the cell cycle inhibitor p21/WAF1 and induction of its proteasomal degradation. This led to increased cell proliferation and provides novel insights into oncogenic kinase-targeted therapeutic intervention for EBV-associated cancers. [PubMed: 25121590]

8. Calderwood MA, Venkatesan K, Xing L, Chase MR, Vazquez A, Holthaus AM, Ewence AE, Li N, Hirozane-Kishikawa T, Hill DE, Vidal M, Kieff E, Johannsen E. Epstein-Barr virus and virus human protein interaction maps. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:7606–7611. [PubMed: 17446270]

9. Cohen JI. Epstein-barr virus vaccines. Clinical & translational immunology. 2015; 4:e32. [PubMed: 25671130]

10. Consortium, E. P. Dunham I, Kundaje A, Aldred SF, Collins PJ, Davis CA, Doyle F, Epstein CB, Frietze S, Harrow J, Kaul R, Khatun J, Lajoie BR, Landt SG, Lee BK, Pauli F, Rosenbloom KR, Sabo P, Safi A, Sanyal A, Shoresh N, Simon JM, Song L, Trinklein ND, Altshuler RC, Birney E, Brown JB, Cheng C, Djebali S, Dong X, Dunham I, Ernst J, Furey TS, Gerstein M, Giardine B, Greven M, Hardison RC, Harris RS, Herrero J, Hoffman MM, Iyer S, Kelllis M, Khatun J, Kheradpour P, Kundaje A, Lassman T, Li Q, Lin X, Marinov GK, Merkel A, Mortazavi A, Parker SC, Reddy TE, Rozowsky J, Schlesinger F, Thurman RE, Wang J, Ward LD, Whitfield TW, Wilder SP, Wu W, Xi HS, Yip KY, Zhuang J, Bernstein BE, Birney E, Dunham I, Green ED, Gunter C, Snyder M, Pazin MJ, Lowdon RF, Dillon LA, Adams LB, Kelly CJ, Zhang J, Wexler JR, Green ED, Good PJ, Feingold EA, Bernstein BE, Birney E, Crawford GE, Dekker J, Elinitski L, Farnham PJ, Gerstein M, Giddings MC, Gingeras TR, Green ED, Guigo R, Hardison RC, Hubbard TJ, Kellis M, Kent WJ, Lieb JD, Margulies EH, Myers RM, Snyder M, Starnatoyannopoulos JA, et al. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012; 489:57–74. [PubMed: 22955616]

11. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012; 485:376–380. [PubMed: 22495300]

12. Frappier L. EBNA1 and host factors in Epstein-Barr virus latent DNA replication. Current opinion in virology. 2012; 2:733–739. [PubMed: 23031715]

13. Gewurz BE, Mar JC, Padi M, Zhao B, Shinners NP, Takasaki K, Bedoya E, Zou JY, Cahir-McFarland E, Quackenbush J, Kieff E. Canonical NF-kappaB activation is essential for Epstein-Barr virus latent membrane protein 1 TES2/CTAR2 gene regulation. Journal of virology. 2011; 85:6764–6773. [PubMed: 21543491]

14. Greenfeld H, Takasaki K, Walsh MJ, Ersing I, Bernhardt K, Ma Y, Fu B, Ashbaugh CW, Cabo J, Mollo SB, Zhou H, Li S, Gewurz BE. TRAF1 Coordinates Polyubiquitin Signaling to Enhance Epstein-Barr Virus LMP1-Mediated Growth and Survival Pathway Activation. PLoS pathogens. 2015; 11:e1004890. [PubMed: 25996949]

15. Halder S, Murakami M, Verma SC, Kumar P, Yi F, Robertson ES. Early events associated with infection of Epstein-Barr virus infection of primary B-cells. PloS one. 2009; 4:e7214. [PubMed: 19784370]

16**. Harth-Hertle ML, Scholz BA, Erhard F, Glaser LV, Dolken L, Zimmer R, Kempkes B. Inactivation of Intergenic Enhancers by EBNA3A Initiates and Maintains Polycomb Signatures across a Chromatin Domain Encoding CXCL10 and CXCL9. PLoS Pathog. 2013; 9:e1003638. This study showed that EBNA3A mediated repression requires the action of RBP-Jk/CBF1 and the EBNA3A competes for binding with EBNA2 for RBP-Jk/CBF1 access to sites on the genome. Additionally, the study also demonstrated that transcriptional silencing induced by EBNA3A precedes the appearance of known repressive PcG marks and that these events are triggered by the loss of enhancer activity. [PubMed: 24068939]

17. Hertle ML, Popp C, Petermann S, Maier S, Kremmer E, Lang R, Mages J, Kempkes B. Differential gene expression patterns of EBV infected EBNA-3A positive and negative human B lymphocytes. PLoS Pathog. 2009; 5:e1000506. [PubMed: 19578441]

18. Houldcroft CJ, Kellam P. Host genetics of Epstein-Barr virus infection, latency and disease. Reviews in medical virology. 2015; 25:71–84. [PubMed: 25430668]

19. Jiang S, Willox B, Zhou H, Holthaus AM, Wang A, Shi TT, Maruo S, Kharchenko PV, Johannsen EC, Kieff E, Zhao B. Epstein-Barr Virus Nuclear Antigen 3C binds to BATF/IRF4 or SPI1/IRF4 composite sites and recruits Sin3A to repress CDKN2A. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111:421–426. [PubMed: 24344258]

20. Kang MS, Kieff E. Epstein-Barr virus latent genes. Experimental & molecular medicine. 2015; 47:e131. [PubMed: 25613728]

21. Kaye KM, Devergne O, Harada JN, Izumi KM, Yalamanchili R, Kieff E, Mosialos G. Tumor necrosis factor receptor associated factor 2 is a mediator of NF-kappa B activation by latent infection membrane protein 1, the Epstein-Barr virus transforming protein. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93:11085–11090. [PubMed: 8855313]

22. Knight JS, Sharma N, Robertson ES. SCFSkp2 complex targeted by Epstein-Barr virus essential nuclear antigen. Molecular and cellular biology. 2005; 25:1749–1763. [PubMed: 15713632]

23. Kwok H, Wu CW, Palser AL, Kellam P, Sham PC, Kwong DL, Chiang AK. Genomic diversity of Epstein-Barr virus genomes isolated from primary nasopharyngeal carcinoma biopsy samples. Journal of virology. 2014; 88:10662–10672. [PubMed: 24991008]

24**. Lee N, Moss WN, Yario TA, Steitz JA. EBV noncoding RNA binds nascent RNA to drive host PAX5 to viral DNA. Cell. 2015; 160:607–618. This group for the first time showed that EBER2 of EBV interacts with Pax5 and is required for localization to the terminal repeats. This strongly supports a role for EBERS and Pax5 in regulating viral replication. Importantly, they showed that this is evolutionarily conserved in the related primate viruses. [PubMed: 25662012]

25. Longenecker, RM., Kieff, E., Cohen, JI. Epstein-Barr virus. In: Knipe, DM.Howley, PM.Cohen, JI.Griffin, DE.Lamb, RA.Martin, MA.Racianello, VR., Roizman, B., editors. Fields Virology. 6. Vol. 2. Lippincott Williams and Wilkins; Philadelphia: 2013. p. 1898-1959.

26. Longnecker R. Epstein-Barr virus latency: LMP2, a regulator or means for Epstein-Barr virus persistence? Advances in cancer research. 2000; 79:175–200. [PubMed: 10818681]

27**. Ma SD, Xu X, Plowshay J, Ranheim EA, Burlingham WJ, Jensen JL, Asimakopoulos F, Tang W, Gulley ML, Cesarman E, Gumperz JE, Kenney SC. LMP1-deficient Epstein-Barr virus mutant requires T cells for lymphomagenesis. The Journal of clinical investigation. 2015; 125:304–315. A LMP1 deletion recombinant virus showed an ability to drive lymphomagenesis in animals but required the contribution of the CD4 T-cells. The implications here are important for the role of LMP1 in driving lymphoma development in vivo and suggests that T-cells can provide signaling activities in the absence of LMP1. [PubMed: 25485679]

28. McClellan MJ, Khasnis S, Wood CD, Palermo RD, Schlick SN, Kanhere AS, Jenner RG, West MJ. Downregulation of integrin receptor-signaling genes by Epstein-Barr virus EBNA 3C via promoter-proximal and -distal binding elements. Journal of virology. 2012; 86:5165–5178. [PubMed: 22357270]

29. McClellan MJ, Wood CD, Ojeniyi O, Cooper TJ, Kanhere A, Arvey A, Webb HM, Palermo RD, Harth-Hertle ML, Kempkes B, Jenner RG, West MJ. Modulation of enhancer looping and differential gene targeting by Epstein-Barr virus transcription factors directs cellular reprogramming. PLoS pathogens. 2013; 9:e1003636. [PubMed: 24068937]

30. Miller CL, Lee JH, Kieff E, Longnecker R. An integral membrane protein (LMP2) blocks reactivation of Epstein-Barr virus from latency following surface immunoglobulin crosslinking. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91:772–776. [PubMed: 8290598]

31. Palser AL, Grayson NE, White RE, Corton C, Correia S, Ba Abdullah MM, Watson SJ, Cotten M, Arrand JR, Murray PG, Allday MJ, Rickinson AB, Young LS, Farrell PJ, Kellam P. Genome diversity of epstein-barr virus from multiple tumor types and normal infection. Journal of virology. 2015; 89:5222–5237. [PubMed: 25787276]

32. Paschos K, Parker GA, Watanatanasup E, White RE, Allday MJ. BIM promoter directly targeted by EBNA3C in polycomb-mediated repression by EBV. Nucleic acids research. 2012; 40:7233–7246. [PubMed: 22584624]

33. Portal D, Zhou H, Zhao B, Kharchenko PV, Lowry E, Wong L, Quackenbush J, Holloway D, Jiang S, Lu Y, Kieff E. Epstein-Barr virus nuclear antigen leader protein localizes to promoters and enhancers with cell transcription factors and EBNA2. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:18537–18542. [PubMed: 24167291]

34. Price AM, Luftig MA. To be or not IIb: a multi-step process for Epstein-Barr virus latency establishment and consequences for B cell tumorigenesis. PLoS Pathog. 2015; 11:e1004656. [PubMed: 25790223]

35. Price AM, Tourigny JP, Forte E, Salinas RE, Dave SS, Luftig MA. Analysis of Epstein-Barr virus-regulated host gene expression changes through primary B-cell outgrowth reveals delayed kinetics of latent membrane protein 1-mediated NF-kappaB activation. Journal of virology. 2012; 86:11096–11106. [PubMed: 22855490]

36. Rickinson AB. Co-infections, inflammation and oncogenesis: future directions for EBV research. Seminars in cancer biology. 2014; 26:99–115. [PubMed: 24751797]

37. Saha A, Halder S, Upadhyay SK, Lu J, Kumar P, Murakami M, Cai Q, Robertson ES. Epstein-Barr virus nuclear antigen 3C facilitates G1-S transition by stabilizing and enhancing the function of cyclin D1. PLoS pathogens. 2011; 7:e1001275. [PubMed: 21347341]

38. Saha A, Lu J, Morizur L, Upadhyay SK, Aj MP, Robertson ES. E2F1 mediated apoptosis induced by the DNA damage response is blocked by EBV nuclear antigen 3C in lymphoblastoid cells. PLoS pathogens. 2012; 8:e1002573. [PubMed: 22438805]

39. Saha A, Robertson ES. Epstein-Barr virus-associated B-cell lymphomas: pathogenesis and clinical outcomes. Clinical cancer research: an official journal of the American Association for Cancer Research. 2011; 17:3056–3063. [PubMed: 21372216]

40. Saha A, Robertson ES. Impact of EBV essential nuclear protein EBNA-3C on B-cell proliferation and apoptosis. Future microbiology. 2013; 8:323–352. [PubMed: 23464371]

41. Schmidt SC, Jiang S, Zhou H, Willox B, Holthaus AM, Kharchenko PV, Johannsen EC, Kieff E, Zhao B. Epstein-Barr virus nuclear antigen 3A partially coincides with EBNA3C genome-wide and is tethered to DNA through BATF complexes. Proceedings of the National Academy of Sciences of the United States of America. 2015; 112:554–559. [PubMed: 25540416]

42. Selvaraj S, RD J, Bansal V, Ren B. Whole-genome haplotype reconstruction using proximity-ligation and shotgun sequencing. Nature biotechnology. 2013; 31:1111–1118.

43. Sharp TV, Raine DA, Gewert DR, Joshi B, Jagus R, Clemens MJ. Activation of the interferon-inducible (2′-5′) oligoadenylate synthetase by the Epstein-Barr virus RNA, EBER-1. Virology. 1999; 257:303–313. [PubMed: 10329541]

44. Sharp TV, Schwemmle M, Jeffrey I, Laing K, Mellor H, Proud CG, Hilse K, Clemens MJ. Comparative analysis of the regulation of the interferon-inducible protein kinase PKR by Epstein-Barr virus RNAs EBER-1 and EBER-2 and adenovirus VAI RNA. Nucleic acids research. 1993; 21:4483–4490. [PubMed: 7901835]

45**. Skalska L, White RE, Parker GA, Turro E, Sinclair AJ, Paschos K, Allday MJ. Induction of p16(INK4a) is the major barrier to proliferation when Epstein-Barr virus (EBV) transforms primary B cells into lymphoblastoid cell lines. PLoS Pathog. 2013; 9:e1003187. This study revealed that EBNA3C is necessary to overcome EBV increase in p16 expression levels and a block to proliferation early after infection of B-cells. The study showed that if the cells are p16 null then a functional EBNA3C is not important for outgrowth of EBV transformed Lymphoblastoid cell lines. [PubMed: 23436997]

46. Swaminathan S, Tomkinson B, Kieff E. Recombinant Epstein-Barr virus with small RNA (EBER) genes deleted transforms lymphocytes and replicates in vitro. Proceedings of the National Academy of Sciences of the United States of America. 1991; 88:1546–1550. [PubMed: 1847527]

47. Thorley-Lawson DA, Hawkins JB, Tracy SI, Shapiro M. The pathogenesis of Epstein-Barr virus persistent infection. Current opinion in virology. 2013; 3:227–232. [PubMed: 23683686]

48. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. Nature reviews Cancer. 2004; 4:757–768. [PubMed: 15510157]

49. Zhao B, Zou J, Wang H, Johannsen E, Peng CW, Quackenbush J, Mar JC, Morton CC, Freedman ML, Blacklow SC, Aster JC, Bernstein BE, Kieff E. Epstein-Barr virus exploits intrinsic B-lymphocyte transcription programs to achieve immortal cell growth. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:14902–14907. [PubMed: 21746931]

50**. Zhou H, Schmidt SC, Jiang S, Willox B, Bernhardt K, Liang J, Johannsen EC, Kharchenko P, Gewurz BE, Kieff E, Zhao B. Epstein-Barr virus oncoprotein super-enhancers control B cell growth. Cell host & microbe. 2015; 17:205–216. This study identified 4 EBV antigens and 5 subunits of NF-KB that co-occupy approximately 1,800 enhancer sites. They went on to identify 187 of these sites as EBV super-enhancers whcih include teh c-myc and bcl2 genes. [PubMed: 25639793]

Highlights

EBV is an oncogenic gammaherpesvirus that transforms human primary B-cells.

EBV encoded transcription factors assemble at primed enhancers to activate cell promoters.

Phosphorylation and degradation of p21 mediated by the Pim-1 kinase is enhanced by EBNA3C.

Growth and survival of infected B cells by latent membrane protein 1 is induced by LUBAC and TRAF1.

Latent membrane protein 2 regulates the autophagosome to block death of infected cells.

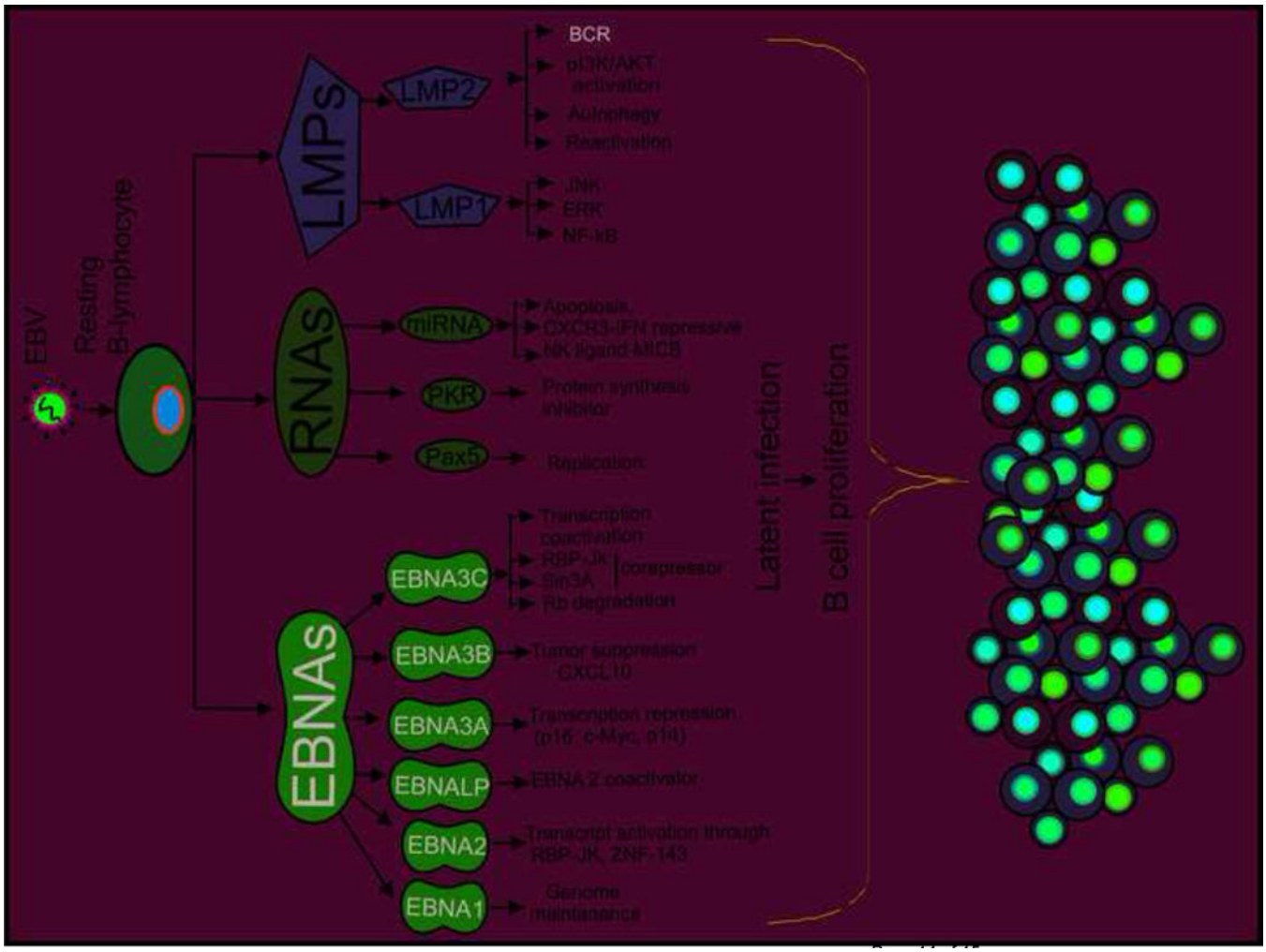

Figure 1.

The schematic shows a comprehensive view of the many components of the latent life cycle of EBV involving the latent membrane proteins (LMPs) and nuclear antigens (EBNAs) as well as the small RNAs encoded by EBV. EBV infection of resting B-lymphocytes lead to expression of the complete set of latent transcripts. The LMPs are involved in dysregulating a number of number of major cellular pathways including JNK, PI3K and NF-KB as well as reactivation of the virus. The small RNAs are now shown to be involved in a number of different activities including replication and apoptosis. EBNAs target a wide range of cellular processes from gene expression, genome maintenance, tumor suppression and cell cycle regulation. These activities contribute the EBV mediated B-lymphocyte transformation of infected cells and suggest that a coordinated set of these activities are critical for driving the linked pathologies.