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Review

Regulation of the latent-lytic switch in Epstein–Barr virus

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ABSTRACT

Epstein–Barr virus (EBV) infection contributes to the development of several different types of human malignancy, including Burkitt lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma. As a herpesvirus, EBV can establish latent or lytic infection in cells. EBV-positive tumors are composed almost exclusively of cells with latent EBV infection. Strategies for inducing the lytic form of EBV infection in tumor cells are being investigated as a potential therapy for EBV-positive tumors. In this article, we review how cellular and viral proteins regulate the latent-lytic EBV switch in infected B cells and epithelial cells, and discuss how harnessing lytic viral reactivation might be used therapeutically.

1. Introduction

Epstein–Barr virus (EBV) is a human herpesvirus that causes infectious mononucleosis. It is also associated with the development of certain malignancies, including African Burkitt lymphomas (BL), B-cell lymphomas of immunocompromised patients, nasopharyngeal carcinomas (NPC), Hodgkin's disease, and, occasionally, with T-cell lymphomas and gastric cancers [1]. Like all herpesviruses, EBV can infect cells in either latent or lytic forms [1,2]. Latent infection occurs in memory B cells, allowing the virus to evade the host immune response and to persist indefinitely within humans [1,2]. Regardless of cell type, all EBV-associated malignancies largely consist of latently infected cells in which EBV-encoded transforming proteins and non-coding RNAs are expressed. The presence of a limited number of lytically infected cells may enhance tumor growth through release of growth factors and immunosuppressive cytokines [3–5].

Lytic EBV infection is essential for production of infectious viral particles, enabling virus transmission from cell to cell and host to host [1,2]. Lytic infection occurs in differentiated oropharyngeal epithelial cells [6,7], and tonsillar plasma cells [8]. *In vitro* studies indicate that B-cell receptor (BCR) stimulation [9], hypoxia [10], and transforming growth factor-beta (TGF-β) [11–13] can also induce lytic replication under some circumstances. EBV's ability to remain latent in memory B cells, yet lytically reactivate under appropriate circumstances, likely explains its near universality in humans. Furthermore, by inducing lytic reactivation in EBV-positive tumors, one could potentially selectively kill EBV-positive malignant cells.

Here, we highlight some recent findings relating to how cellular and viral factors promote or inhibit EBV reactivation and discuss how "lytic induction therapy" might be used to treat patients with EBV-positive tumors. We refer readers to prior review articles for coverage of the older literature on these and related topics [2,14–22].

2. EBV lytic reactivation from latent infection

2.1. Overview

In latently infected cells, the double-stranded DNA genome of EBV is maintained as a nuclear episome replicated once per cell cycle by the host DNA polymerase. It is usually highly methylated, existing in a repressive chromatin structure. Following reactivation, the lytic genes of EBV are expressed in a temporally regulated manner. The first ones transcribed are the viral immediate-early (IE) lytic genes, *BZLF1* and *BRLF1* (Fig. 1A). They encode the transcription factors, Z (aka Z, ZTA, ZEBRA) and R (aka R, RTA), respectively. Neither *BZLF1* nor *BRLF1* is expressed in latently infected cells due to silencing by multiple cellular transcriptional repressors. The promoters of these genes (Zp and Rp, respectively) are initially activated by cellular transcription factors (Fig. 1B and C). Subsequently, the Z and R proteins activate both their own and one another's promoters to greatly amplify their lytic-inducing effects. They then cooperatively activate the promoters of early (E) lytic genes that encode the viral replication proteins. Following viral genome replication, the late (L) viral genes are expressed. The latter encode

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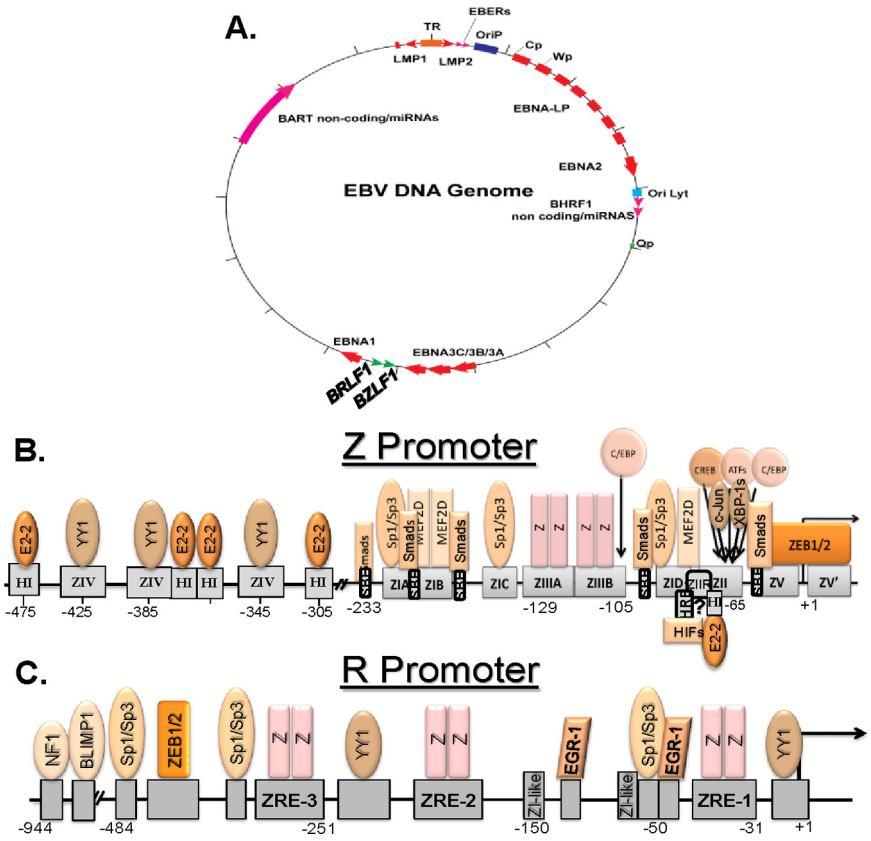

Fig. 1. Schematics (not drawn to accurate scale) showing (A) the locations of the *BZLF1* and *BRLF1* genes within the context of the EBV genome, and (B and C) factors known to play roles in regulating transcription from the promoters of these genes, Zp and Rp, respectively. *Cis*-acting elements are indicated by gray rectangles, with their corresponding *trans*-acting factors shown directly above or below them. ZRE, Z-responsive element; SBE, Smads-responsive element; and HRE, hypoxia-responsive element.

structural proteins required for viral genome encapsidation into infectious virion particles.

2.2. Z-mediated lytic reactivation

In most EBV-positive cell lines, synthesis of Z protein is sufficient to induce the switch from a latent to lytic form of viral infection. Z, a member of the bZip family, binds as a homodimer to AP-1-like motifs known as Z-responsive elements (ZREs). Z protein contains an amino-terminal transactivator domain, a DNA-binding domain homologous to the basic DNA-binding domains of c-Jun and c-fos, and a carboxy-terminal bZip homodimerization domain [23]. Z interacts directly with histone acetylating complexes such as CBP and p300 and the general transcription factors TFIID and TFIIA. During viral reactivation (in cells in which the EBV genome is highly methylated), Z initially activates transcription from Rp. Z and R then activate transcription from multiple early lytic viral promoters which often contain binding sites for both [25]. They are both required for expression of many, but not all, of the early-lytic genes within the context of the intact viral genome [24].

Z also plays an important role in lytic EBV DNA replication, binding directly to a series of essential ZRE sites located within the lytic origin of replication, *oriLyt*. The role(s) Z plays in mediating lytic EBV DNA replication are distinct from its transcriptional functions [26], with direct interactions between Z and core viral replication proteins likely promoting formation of replication complexes.

2.3. R-mediated lytic reactivation

R can also induce the switch from latent to lytic infection in some EBV-positive cell lines, particularly epithelial cells. The closely related Kaposi's sarcoma herpesvirus (KSHV) exclusively uses its R homolog (ORF50; RTA), rather than a Z-like protein (K8), to disrupt viral latency. R contains an amino-terminal DNA-binding domain, a homodimerization domain, and a carboxy-terminal transcriptional activation domain. It binds directly to GC-rich motifs known as R-responsive elements (RREs) (consensus 5'-GNCCN$_9$GGNG-3') located within the promoters of early lytic genes, functioning as a powerful enhancer when bound to these sites [27]. R directly interacts with both the general transcription factors TBP and TFIIB and the histone acetylases CBP and p300. Data from transient transfection reporter assays indicate that R activates both its own promoter and Zp by indirect mechanisms involving protein interactions with the Sp1, MCAF1 and Oct-1 transcription factors, and induction of cellular kinases that activate the c-Jun and ATF-2 transcription factors [28–32]. However, given the powerful enhancer activity of R-bound RREs, we speculate that a primary mechanism by which R activates Rp/Zp transcription in the context of the intact viral genome may well be through direct binding to RREs located potentially thousands of base pairs away from transcription initiation sites (and, thus, not present in the reporter constructs used in the transient transfection assays) [27].

At least two EBV-encoded proteins differentially regulate the ability of R to disrupt viral latency. The early-lytic viral protein Na (encoded by BRRF1) activates phosphorylation of c-Jun and cooperates with R to induce transcription from Zp in the context of the intact viral genome [33,34]. In contrast, the early-lytic viral protein LF2 directly interacts with R, sequestering it in an inactive form in the cytoplasm [35]. Presumably, the opposing effects of BRRF1 and LF2 help to fine-tune the transcriptional effects of R during the various stages of EBV lytic replication.

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