Regulation of Epstein-Barr Virus Lytic Cycle Activation in Malignant and Nonmalignant Disease

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Like all other herpesviruses, the oncogenic human gamma-herpesvirus, Epstein-Barr virus (EBV; human herpesvirus 4) manifests two distinct phases in its life cycle: latency and lytic replication. During latency EBV expresses a limited number of viral genes that are involved in tasks such as stimulating cell proliferation, inhibiting apoptosis, blocking viral lytic replication, and assuring accurate and equal partitioning of the episomal viral genome to daughter cells. Variants of the EBV latent life cycle, which are distinguished by the latent products expressed, are characteristic of each EBV-associated cancer. During the lytic replication phase of the EBV life cycle, many more viral genes, encoding enzymes, and other proteins involved in nucleotide biosynthesis, RNA processing, viral DNA replication, and capsid and viral envelope synthesis, are expressed. Two genes of EBV, BZLF1 and BRLF1, encode transcriptional activator proteins that mediate the switch between latency and the lytic cycle (1–3). Both genes are essential for this switch (4). BZLF1, encoding ZEBRA (Z, EB Replication Activator), is dominant in EBV (5), although the homologue of BRLF1, encoded in open reading frame (ORF) 50, is the dominant lytic switch protein in the related Kaposi’s sarcoma-associated herpesvirus (6). ZEBRA activates expression of Rta (R transactivator), the product of the EBV BRLF1 gene, in all cell backgrounds that have been studied. The capacity of Rta to activate ZEBRA expression is restricted to certain cell backgrounds (7,8).

The molecular events involved in the switch between the latent and lytic replication phases of the EBV life cycle can be studied in cultured lymphoblastoid or lymphoma cell lines. Transfection of a ZEBRA expression vector is sufficient to activate the lytic cycle. A number of other exogenous stimuli are competent to trigger the switch; these include protein kinase C agonists such as phorbol esters, histone deacetylase inhibitors such as n-butyrate and Trichostatin A, anti-immunoglobulin, calcium ionophores, transforming growth factor-beta (TGF-β),...
ments have been divided into three classes, termed ZI, ZII, and ZIII sites. The ZI sites bind the cellular proteins Sp1 and Sp3 (ZIA, ZIC, and ZID), or both (ZIA and ZID). MEF2D in turn binds class II histone deacytelases (11). This interaction may be crucial in repression of Zp during latency. There is direct evidence in some cell backgrounds, such as the Akata Burkitt lymphoma cell line, that Zp is repressed by histones in chromatin (12). The ZI Sp1 and Sp3 binding sites are involved in mediating the capacity of phorbol esters and anti-immunoglobulin to induce the EBV lytic cycle (13). The ZII site, which binds transcriptional activators of the activating transcription factor family, is also an important mediator of the response downstream of protein kinase C agonists (14). The two ZIII sites bind ZEBRA protein itself and are considered to be responsible for the ability of ZEBRA to autostimulate its own promoter (15). However, Zp autostimulation does not take place in all lymphoma cell backgrounds (16,17). In addition to global repression by chromatin, perhaps mediated through the MEF2D binding sites, Zp appears to be inhibited by specific repressors whose molecular characterization and mechanism of action is still in early stages of investigation (18–20).

In this issue of the Journal, Gutiérrez et al. (21) describe molecular epidemiologic studies of three polymorphic variants within the 200-nt Zp. One variant, termed Zp-P (prototype) is identical in sequence to the prototype EBV strain B95–8 (22). A second variant, termed Zp-V3, contains three point changes: A to G at position –141, A to G at position –106, and T to G at position –100 relative to the start of BZLF1 transcription. The third variant, Zp-V4, contains all of the Zp-V3 substitutions plus a fourth substitution, T to G at position –196. Remarkably, all of these single nucleotide polymorphisms (SNPs) are within or close to the characterized regulatory elements of Zp. Two of them lie directly within ZI sites (–196 within ZIA and –141 within ZIC); the –106 variant is located four nucleotides downstream of the ZIIIb site and the –100 variant, two nucleotides upstream of the ZID site.

The most striking result of the study was the strict correlation between the Zp-V4 polymorphism and tumor or non-tumor specimens. The Zp-V4 variant was absent from 52 tissue samples obtained from EBV-associated malignant tumors such as non-Hodgkin’s lymphoma, post-transplant lymphoproliferative disease, and nasopharyngeal cancer. Zp polymorphisms in the tumors were approximately equally divided between Zp-P and Zp-V3; the Zp-V3 variants were uniformly type B, and the Zp variants were predominantly type A EBV. By contrast, the Zp-V4 variant was found in every sample from 13 patients with nonmalignant EBV-associated disease, such as chronic active EBV infection and infectious mononucleosis (some of these were co-infected with Zp-P variant virus), and from all 40 individuals who were healthy EBV carriers. Because the Zp-V3 and Zp-V4 variants differ at a single nucleotide (T to C at position –196 in the ZIA site), the implication of this finding is that this one SNP spells the difference between benign and malignant EBV-associated disease. A corollary, favored by the authors but not directly examined in the study, is that the –196 SNP promotes lytic EBV replication, which exerts an antineoplastic effect. Once in its fully lytic phase, EBV, like other herpesviruses, kills its host cell. Future experiments will surely be directed toward understanding the consequences of the –196 SNP. For example, does it enhance or inhibit binding or protein–protein interactions of Sp1, Sp3, or MEF2D at the ZIA site? When present in the context of a complete viral genome, does this SNP alone promote or inhibit spontaneous or induced lytic cycle reactivation or must it be accompanied by the other SNPs in Zp-V3?

Although the result is extremely provocative, a few words of caution are merited. A more extensive molecular epidemiologic study is required in which samples from malignant disease and healthy carriers are meticulously matched for geographic origin and for family exposure. It would also be helpful to examine EBV-infected tumor and non-tumor cells from the same individual. All the samples should be analyzed by DNA sequencing because polymerase chain reaction–single-strand conformation polymorphism, although useful as a screening test, is not as specific as sequencing. Other regions of the genome present in tumor and non-tumor tissue should be compared to learn whether the Zp polymorphisms alone specifically segregate with tumor tissues. The history of EBV research is replete with attempts to identify viral variants that would segregate specifically between benign and malignant EBV-associated disease. A corollary, favored by the authors but not directly examined in the study, is that the –196 SNP promotes lytic EBV replication, which exerts an antineoplastic effect. Once in its fully lytic phase, EBV, like other herpesviruses, kills its host cell. Future experiments will surely be directed toward understanding the consequences of the –196 SNP. For example, does it enhance or inhibit binding or protein–protein interactions of Sp1, Sp3, or MEF2D at the ZIA site? When present in the context of a complete viral genome, does this SNP alone promote or inhibit spontaneous or induced lytic cycle reactivation or must it be accompanied by the other SNPs in Zp-V3?

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by disease. Although there have been some promising leads, none of them has yet explained the perplexing diversity of EBV-associated diseases.

Lytic cycle replication of EBV is an essential, although perhaps indirect, component of the pathogenesis of EBV-associated cancers. The lytic cycle permits the virus to spread within a population of individuals and from cell to cell. Lytic cycle events are the most promising targets for vaccine development. The article by Gutiérrez et al. (21) raises the intriguing possibility that regulation of BZLF1 gene expression plays a more immediate role in determining the oncogenic outcome of virus infection by inhibiting or promoting the latency-to-lytic-cycle switch.

REFERENCES