Inhibition of the Epstein–Barr virus lytic cycle by Zta-targeted RNA interference

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Epstein–Barr virus (EBV) reactivation into the lytic cycle plays certain roles in the development of EBV-associated diseases, so an effective strategy to block the viral lytic cycle may be of value to reduce the disease risk or to improve the clinical outcome. This study examined whether the EBV lytic cycle could be inhibited using RNA interference (RNAi) directed against the essential viral gene Zta. In cases of EBV reactivation triggered by chemicals or by exogenous Rta, Zta-targeted RNAi prevented the induction of Zta and its downstream genes and further blocked the lytic replication of viral genomes. This antiviral effect of RNAi was not likely to be mediated by activation of the interferon pathway, as phosphorylation of STAT1 was not induced. In addition, novel EBV-infected epithelial cells showing constitutive activation of the lytic cycle were cloned; such established lytic infection was also suppressed by Zta-targeted RNAi. These results indicate that RNAi can be used to inhibit the EBV lytic cycle effectively in vitro and could also be of potential use to develop anti-EBV treatments.

INTRODUCTION

Epstein–Barr virus (EBV), a gammaherpesvirus associated with several human diseases, has two phases in its life cycle: latent and lytic stages (Kieff & Rickinson, 2001). During EBV latency, only a few so-called latent genes are expressed, whereas an expression cascade of numerous lytic genes occurs following reactivation of the lytic cycle. In latency, the copy number of viral DNA is maintained at a relatively low level and no virion is produced but, in the EBV lytic cycle, there is amplification of viral genomes and production of virus particles (Kieff & Rickinson, 2001; Metzenberg, 1990).

The majority of EBV infection in vivo is latent and it is this type of infection that is observed in peripheral B lymphocytes of healthy carriers and in most tumour cells of EBV-associated cancers such as nasopharyngeal carcinoma (NPC), Hodgkin’s disease (HD) and endemic Burkitt’s lymphoma (Rickinson & Kieff, 2001). Intriguingly, several clues indicate that EBV reactivation into the lytic cycle may play a role in the pathogenesis of these malignancies. Elevated antibody titres against EBV lytic antigens and increased viral DNA load in serum/plasma, two parameters that represent EBV reactivation in vivo, correlate with advanced cancer stages, poor prognosis or tumour recurrence after therapy (de-Vathaire et al., 1988; Henle et al., 1969, 1977; Henle & Henle, 1976; Lei et al., 2000; Levine et al., 1971; Lo, 2001). Serological studies further suggest that EBV reactivation may occur months or years before the clinical diagnosis of NPC, HD and endemic Burkitt’s lymphoma, serving as a risk factor of cancer development (Chien et al., 2001; Geser et al., 1982; Mueller et al., 1989; Zeng et al., 1985). Another notable clue comes from in vitro studies, in which the EBV lytic cycle was activated by extracts of some foodstuffs or plants that were identified as dietary or environmental risk factors of NPC or endemic Burkitt’s lymphoma (Bouvier et al., 1995; MacNeil et al., 2003; Shao et al., 1988).

The aetiological role of the EBV lytic cycle is further identified in the disease oral hairy leukoplakia (OHL), a tongue lesion with epithelial hyperplasia in immunodeficient patients (Rickinson & Kieff, 2001; Triantos et al., 1997). OHL is characterized by an unusual activation of the EBV lytic cycle, so that abundant EBV lytic proteins, viral genomes and virus particles are detected in the squamous epithelial cells (Becker et al., 1991; Greenspan et al., 1985; Young et al., 1991). Inhibition of the productive EBV replication by antiviral agents, such as acyclovir, results in effective resolution of OHL, indicating that EBV lytic replication is necessary for the pathogenesis of the lesion (Resnick et al., 1988; Triantos et al., 1997). However, such treatment is not always satisfactory, because the EBV lytic cycle and OHL lesions frequently recur after withdrawal of the drugs (Resnick et al., 1988; Triantos et al., 1997; Walling et al., 2003).

As EBV reactivation and productive replication are involved in EBV-related diseases, development of an effective strategy...
to inhibit the EBV lytic cycle may be of value in reducing the disease risk or improving clinical outcome. One attractive approach is RNA interference (RNAi), in which sequence-specific RNA degradation leading to gene silencing is directed by a short RNA duplex, small interfering RNA (siRNA) (Dykxhoorn et al., 2003; Gitlin & Andino, 2003). The siRNA-mediated inhibition of virus replication is successful in some viral systems that exhibit productive infection (Ge et al., 2003; Gitlin et al., 2002; Jia & Sun, 2003; Novina et al., 2002). However, such a permissive system for EBV productive replication has not been available, in that EBV infection in vitro is predominantly restricted to latency (Rickinson & Kieff, 2001). The EBV lytic cycle can be conditionally activated in vitro, but it has still not been tested whether RNAi can prevent the viral switch from latency to lytic replication.

Based on the following reasons, our first RNAi target gene for inhibiting EBV reactivation is Zta, an immediate-early gene of the lytic cycle. (i) Exogenous stimuli triggering EBV reactivation induce Zta expression (Mellinghoff et al., 1991). Being a key transcriptional activator, Zta protein is sufficient to disrupt EBV latency (Grogan et al., 1987). (ii) A study of a Zta-deleted EBV mutant showed that Zta is essential for full expression of lytic genes and for viral DNA replication (Feederle et al., 2000). (iii) Theoretically, Zta-targeted RNAi will knock down not only Zta but also endogenous Rta, another essential transcriptional activator of the lytic cycle, because endogenous Rta is expressed from the Rta/Zta bicistronic mRNAs that should be degraded by Zta-specific RNAi (Feederle et al., 2000; Manet et al., 1989). (iv) In the initial step of EBV reactivation, Zta and Rta autostimulate their own expression, reciprocally activate each other and cooperatively induce the downstream lytic cascade (Adamson et al., 2000; Holley-Guthrie et al., 1990; Liu & Speck, 2003; Ragoczy et al., 1998). Therefore, Zta-targeted RNAi may block the positive feedback loop at the beginning of the lytic cycle.

In this study, we examined the potential of Zta-targeted RNAi to inhibit the EBV lytic cycle in two situations: one was EBV reactivation induced by exogenous stimuli and the other was a novel infection state showing constitutive activation of the EBV lytic cycle in epithelial cells.

**METHODS**

**Cells.** NA is an EBV-positive NPC cell line previously generated by in vitro infection of NPC-TW01 cells with recombinant Akata EBV (Chang et al., 1999). To establish EBV-infected 293A cells, parental 293 cells were infected with recombinant Akata EBV and selected with G418 as described previously (Chang et al., 1999). The 293A cell clones were further isolated by limiting dilution and single-cell cloning. EBV-negative 293-pZip cells were generated by transfection of 293 cells with the pZipNeoSV(X)1 plasmid expressing a G418-resistance gene (Chang et al., 1998). All the cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum.

**Plasmids.** The pSUPER plasmid was described by Brummelkamp et al. (2002). siRNA-expressing plasmids were constructed by cloning siRNA sequences into pSUPER via BglII and HindIII sites. siRNAs targeted against Zta and green fluorescence protein (GFP) were designed using a program available online (https://www.genscript.com/ssl-bin/app/rnai) and the top-ranked sequence for each gene was chosen in this study. The siRNA sequences were further subjected to a BLAST search against human genome and EST databases to ensure that no human gene was targeted. The Zta-targeted siRNA, siZ1, is directed against the Zta sequence 5'-CAACAGCT-AGCAGACATTG-3' (nucleotides 415-433 downstream of the start codon). The targeted Zta sequence is conserved among Akata, B95-8 and P3HR-1 strains of EBV. The predicted structure of siZ1 after transcription and processing in cells is shown in Fig. 1(a). The sequence of GFP-targeted siRNA, siGFP, is 5'-GCTGACCCCTGAGTTTCATCTG-3'. The plasmids expressing Zta and GFP were derived from the pBc/CMV plasmid (Lu et al., 2000). The Rta-expressing plasmid RTS15 was described by Ragoczy et al. (1998).

**Antibodies.** mAbs used for detection of EBV lytic proteins included 4F10 (anti-Zta; Tsai et al., 1997), 467 (anti-Rta), 88A9 (anti-BMRF1; Tsai et al., 1991), 3E8 (anti-BHRF1), 343D12 [anti-125 kDa viral capsid antigen (VCA); Tsai & Glaser, 1991] and 201D6 [anti-membrane antigen (MA); Tsai et al., 1991]. An NPC patient’s serum was used to detect EBV nuclear antigen 1 (EBNA1) proteins (Chang et al., 1999). The anti-GFP antibody was purchased from BD.

**Fig. 1.** Specific knockdown of target genes by siRNAs. (a) Predicted structure of siZ1 after transcription from a pSUPER-based plasmid and intracellular processing (Brummelkamp et al., 2002). It consists of two 21-nt single-stranded RNAs forming a 19 bp duplex with 2-nt 3’ overhangs (Dykxhoorn et al., 2003). (b) Specific inhibition of gene expression by siRNA. Transfection of plasmids expressing Zta, GFP or Rta was in combination with transfection of the control pSUPER vector or pSUPER-derived plasmids expressing siZ1 or siGFP in 293 cells. Gene expression was detected by the immunoblotting assay using mAbs against Zta, Rta, GFP or β-actin.
Biosciences Clontech and the anti-β-actin antibody was from Sigma. Specific rabbit antibodies were used to detect total STAT1 proteins or phosphorylated STAT1 (at tyrosine 701) (Cell Signalling).

Expression of siRNA and induction of the EBV lytic cycle. In the experiment shown in Fig. 1(b), 3 µg pSUPER-derived siRNA-producing plasmids were co-transfected with 3 µg plasmids expressing GFP, Zta or Rta into 293 cells using Lipofectamine 2000 transfection reagent (Invitrogen). For siRNA-mediated inhibition of EBV reactivation, NA cells were pre-transfected with 5 µg siRNA-producing plasmids for 48 h and then either treated with 12-O-tetradecanoylphorbol-13-acetate (TPA; 40 ng ml⁻¹) or provided in a previous study (Lo et al., 1999). EBV genome was performed following our previous protocol (Lu et al., 2000). In each lane, 15 µg total RNA was electrophoresed and hybridized with 32P-labelled DNA probes recognizing Zta or the internal control GAPDH.

Detection of viral and cellular RNAs. The Northern blotting assay was carried out as described previously (Lu et al., 2000). The positive control for expression of EBV lytic proteins was the protein lysate of NA cells treated with TPA and n-butylate for 48 h (Chang et al., 1999). The positive control for activation of the interferon pathway was the protein lysate of NA cells treated with interferon α (1000 U ml⁻¹) for 10 min. An immunofluorescence assay was used to examine the percentage of cells expressing lytic proteins, as described previously (Chang et al., 1999).

Detection and quantification of EBV DNA. Cells were lysed and digested by proteinase K as described previously and then subjected to the following PCR analysis (Chang et al., 1999). Conventional PCR detection of BamHI W fragments of the EBV genome was performed following our previous protocol (Chang et al., 2002). For quantification of EBV DNA, real-time PCR was used to detect viral DNA using primers and probes provided in a previous study (Lo et al., 1999). We used H2B4 cells harbouring one EBV genome per cell to generate a standard curve for quantification (Chang et al., 2002) and EBV copy number was calculated by comparison with the standard. All samples were tested in duplicate.

Titration of infectious EBV particles. Filtered culture supernatants of transfected cells were subjected to serial twofold dilution and used to infect human peripheral blood mononuclear cells as described previously (Miller & Lipman, 1973). The titre of EBV was determined by its ability to transform primary B lymphocytes into lymphoblastoid cell lines in 4 weeks.

RESULTS

Specific inhibition of gene expression by RNAi

We used a plasmid-based pSUPER system to drive siRNA synthesis in transfected cells (Brummelkamp et al., 2002). The specificity of siRNA-mediated gene silencing was tested in EBV-negative 293 cells that were co-transfected with plasmids expressing siRNA and target genes. Expression of Zta was inhibited only by Zta-targeted siRNA (siZ1) and GFP expression was inhibited only by its specific siRNA (siGFP) (Fig. 1b). The control vector pSUPER did not affect the expression of Zta and GFP, and the level of exogenous Rta was not influenced by either siZ1 or siGFP (Fig. 1b). Therefore, our siRNAs function to knock down specifically their own target genes.

Zta-targeted RNAi inhibited EBV reactivation induced by chemicals

Next, we tested whether siZ1 could prevent EBV reactivation induced by TPA and n-butylate in an EBV-positive NPC cell line, NA (Chang et al., 1999). The cells were transiently transfected with siRNA-expressing plasmids and treated with the chemicals. After chemical induction in the absence of siRNA, three major species of viral transcripts encoding Zta were detected: 1 kb Zta monocistronic mRNA and 4 kb and 3 kb Rta/Zta bicistronic mRNAs (Fig. 2a) (Manet et al., 1989; Mellinhoff et al., 1991). In agreement with expectations, all three transcripts were significantly diminished in the presence of siZ1 (Fig. 2a). Consistently, the protein levels of Zta and Rta induced by the chemicals were apparently decreased by siZ1 (Fig. 2b). We also observed siZ1-mediated reduction of expression of the viral BMRF1 and BHRF1 genes, both of which are downstream lytic genes regulated by Zta and Rta (Fig. 2b) (Cox et al., 1990; Holley-Guthrie et al., 1990). To examine whether siZ1 further suppressed lytic replication of the viral genomes, EBV DNA copies in NA cells were quantified using a real-time PCR method (Lo et al., 1999). Untreated NA cells harboured about 15 copies of EBV DNA per cell on average, while viral genomes increased more than tenfold after 1 day of chemical induction in the absence of siRNA (Fig. 2c). Such viral DNA amplification in the lytic cycle was almost completely blocked by siZ1 (Fig. 2c). EBV reactivation triggered by TPA and n-butylate was inhibited specifically by siZ1-directed RNAi, since the inhibition could not be achieved by either siGFP or the control pSUPER vector (Fig. 2). Meanwhile, siZ1 had no effect on the expression of a latent gene, EBNA1 (Fig. 2b).

Zta-targeted RNAi inhibited Rta-induced EBV reactivation

Previous reports have shown that expression of Rta is sufficient to disrupt EBV latency in several cell lines (Ragoczky et al., 1998; Zalani et al., 1996). Here, we further examined whether siZ1 could block the EBV reactivation induced by ectopic expression of Rta. Exogenous Rta induced the production of Zta-encoded monocistronic and bicistronic mRNAs, the expression of Zta, BMRF1 and BHRF1 proteins and the amplification of EBV DNA copies, indicating that Rta efficiently disrupted EBV latency in NA cells (Fig. 3). The Rta-induced expression of Zta and BMRF1 was significantly diminished by siZ1 but not by siGFP, while siZ1 did not affect exogenous Rta (Fig. 3a, b). Furthermore, siZ1 also prevented Rta-induced amplification of viral genomes (Fig. 3c), indicating that siZ1-mediated inhibition of EBV lytic replication is sustained even in the presence of Rta proteins. On the other hand, Rta-mediated induction of BHRF1 was not
**Fig. 2.** siZ1-mediated inhibition of chemically induced EBV reactivation. NA cells were untransfected (control) or transfected with empty vectors (pSUPER) or plasmids expressing siZ1 or siGFP for 48 h and then treated with (+) or without (−) TPA plus sodium n-butyrate (T/S) for 24 h. (a) Detection of viral transcripts by the Northern blotting assay. Total RNAs were electrophoresed and hybridized with ³²P-labelled DNA probes recognizing Zta or the internal control GAPDH. The predicted mRNAs are indicated. Using the Zta probe, both 1 kb monocistronic Zta mRNAs (Z) and 4 kb and 3 kb Rta/Zta bicistronic mRNAs (R+Z) were detected in the EBV lytic cycle (Manet et al., 1989; Mellinghoff et al., 1991). (b) Immunoblotting analysis of EBV protein expression. Expression of Zta, Rta, BMRF1, BHRF1, EBNA1 and cellular β-actin was examined in the analysis. (c) Quantification of EBV DNA using real-time PCR. Shown are the copy numbers of EBV DNA per cell. Each sample was tested in duplicate and error bars are given.

**Fig. 3.** siZ1-mediated inhibition of Rta-induced EBV reactivation. NA cells were untransfected (control) or transfected with plasmids expressing siZ1 or siGFP for 48 h and then post-transfected with Rta-expressing plasmids (R) or control vectors (V) for 24 h. The detection procedures were the same as outlined in Fig. 2. (a) Detection of viral transcripts by the Northern blotting assay. The predicted mRNAs are indicated. (b) Immunoblotting analysis to detect expression of Zta, Rta, BMRF1, BHRF1, EBNA1 and cellular β-actin. (c) Quantification of EBV DNA by real-time PCR.
affected by siZ1 (Fig. 3b), consistent with a previous study that suggested that the BHRF1 promoter can be activated by Rta alone in the absence of Zta (Cox et al., 1990).

Zta-targeted RNAi did not activate the interferon pathway

Although the action of RNAi was thought to be independent of the interferon pathway, a recent study showed that some siRNAs activate the interferon response (Gitlin & Andino, 2003; Sledz et al., 2003). To examine whether the siZ1-mediated inhibition of EBV reactivation involved the antiviral effect of interferon, we measured the phosphorylation of STAT1, a common signalling event triggered by interferon (Sledz et al., 2003; Stark et al., 1998). While treatment with interferon α induced significant phosphorylation of STAT1 in NA cells, expression of siZ1 caused little enhancement of STAT1 phosphorylation (Fig. 4a). Therefore, the prevention of EBV reactivation by siZ1 was not likely to be mediated by activation of the interferon pathway. In addition, the inhibitory effect of RNAi was not likely to be caused by toxicity to the cells, as expression of siZ1 did not affect the viability of 293 or NA cells (Fig. 4b).

Fig. 4. Lack of effect of Zta-targeted RNAi on STAT1 phosphorylation and cell viability. (a) Examination of STAT1. NA cells were treated the same way as for Fig. 2 (left panel) or Fig. 3 (right panel) and phosphorylated STAT1 (at tyrosine 701) and total STAT1 proteins were detected by immunoblotting analysis. NA cells treated with interferon α were used as the positive control. (b) Examination of cell viability. NA and 293 cells were transfected with indicated plasmids for 48 h and their viability was determined using a trypan blue exclusion method.

Zta-targeted RNAi suppressed constitutive activation of the EBV lytic cycle

As induction of EBV reactivation was effectively inhibited by siZ1, we next asked whether siZ1 could also suppress a lytic cycle that was already in a constitutively activated state, a state like EBV infection in OHL (Greenspan et al., 1985). Although spontaneous activation of the EBV lytic cycle is rare, previous observations indicated that a small proportion of EBV-infected epithelial cells in vitro were permissive for the lytic cycle without exogenous stimuli (Chang et al., 1999; Mauser et al., 2002). In this study, we sought to isolate such permissive cells from EBV-infected 293 cells, 293A, through limiting dilution and single-cell cloning (Fig. 5a). Within the 258 cell clones we examined, EBV infection was restricted to latency in most clones, designated 'latent clones’, as no lytic protein was detected (Fig. 5b, c). Only five cell clones, designated ‘lytic clones’, constitutively expressed lytic proteins such as Zta, Rta, BHRF1, and 125 kDa VCA (Fig. 5b). In 293A-2 and 293A-98 cells, lytic proteins were detected most prominently and about 30% of the cells expressed Zta and BHRF1 (Fig. 5c). The Zta-positive rates in these lytic clones were stable for more than 1 year and cell viability of the lytic clones was indistinguishable from that of latent clones (data not shown). The copy numbers of EBV genomes in latent clones ranged from 1 to 30 copies per cell (Fig. 5d). In contrast, the lytic clones had a higher EBV DNA copy number (ranging from 400 to more than 1000 copies per cell), reflecting the amplification of viral genomes in the lytic cycle (Fig. 5d).

We further examined what level the lytic cycle had reached in the 293A lytic clones in comparison with the maximal level that was induced by ectopic expression of Zta and Rta. Such induction increased by two- to fourfold the positive rates of cells expressing lytic proteins in 293A-2 clones (Fig. 6a, b). Production of infectious EBV was detected in the culture supernatants of untreated lytic clones and virus titres were further raised about 16-fold by exogenous Zta and Rta (Fig. 6c). These results suggest that the EBV lytic cycle in 293A lytic clones is maintained at a moderate level below the maximum that can be potentially activated.

Although the mechanism of constitutive EBV activation in 293A lytic clones remains unclear, these cells were useful for testing the efficacy of siZ1. Two lytic clones, 293A-2 and 293A-98, were subcultured every 4 days and each passage was followed by transfection once with plasmids producing siRNA. Fig. 7(a) shows that repeated transfection with plasmids expressing siZ1 but not siGFP gradually suppressed the levels of lytic proteins. Consistently, the copy numbers of EBV DNA in the lytic clones were also significantly reduced by siZ1 (Fig. 7b). Meanwhile, expression of siZ1 did not affect the level of latent EBNA1 proteins (Fig. 7a).

DISCUSSION

In summary, siZ1-directed RNAi can inhibit not only EBV reactivation induced by chemicals or by exogenous
Rta but also the lytic cycle exhibiting a constitutively active state. This is the first report that the EBV lytic cycle can be effectively blocked by RNAi. One advantage of this study is the use of NA and 293A cells, both of which can be transfected with high efficiency. The efficiency was higher than 90%, as monitored by transfection with GFP-expressing plasmids (data not shown), so a near-complete gene knockdown by RNAi could be achieved in these cells.

Compared with conventional anti-EBV agents such as acyclovir, Zta-targeted RNAi has some advantages. Acyclovir inhibits EBV DNA synthesis but does not cause suppression of immediate-early or early lytic genes such as Zta, Rta and BMRF1, whose expression is independent of viral DNA replication (Takase et al., 1996). These unaffected lytic proteins may facilitate recurrence of the lytic cycle, perhaps explaining the frequent relapse of OHL and EBV reactivation after withdrawal of acyclovir treatment (Resnick et al., 1988; Triantos et al., 1997). Zta-targeted RNAi may prevent the lytic cycle more completely because it blocks the initial and essential step of EBV reactivation, the expression of endogenous Zta and Rta. In addition, RNAi may bring much less unfavourable side-effects than the antiviral drugs as it knocks down gene expression in a sequence-specific manner and may cause little cytotoxicity (Figs 1b and 4b). Our results indicate that RNAi is of potential use in developing a new anti-EBV approach. Before its application to clinical therapy, however, an effective strategy to deliver siRNA into target cells of patients is required. We are testing the feasibility using viral vectors for the delivery of siRNA-expressing genes.

Zta-targeted RNAi is also useful to clarify the roles of Zta and Rta in regulation of downstream lytic genes. A good example provided here is the regulation of BMRF1 and BHRF1. Although the promoters of both genes contain responsive elements for Zta and Rta, they are regulated differentially by the two transactivators (Cox et al., 1990; Holley-Guthrie et al., 1990). In lymphoid cells, the induction of BMRF1 requires cooperation of Zta and Rta, while, in epithelial cells, Zta alone seems sufficient to stimulate BMRF1 expression (Feederle et al., 2000; Ragoczy & Miller, 1999). In this study, siZ1 blocked BMRF1 expression even in the presence of exogenous Rta (Fig. 3b), indicating that Zta is the sole and essential transactivator for the BMRF1 gene in epithelial cells. On the other hand, it has been reported that the BHRF1 promoter could be activated by Rta alone and synergistically enhanced by Zta and Rta (Cox et al., 1990; Ragoczy & Miller, 1999). Our results showed that Rta-mediated induction of BHRF1 was
not affected by siZ1 (Fig. 3b), suggesting that expression of BHRF1 is regulated mainly by Rta and independent of Zta in NA cells. This study indicates that use of siRNAs targeted against lytic genes can be a feasible approach to analyse how the viral expression cascade is regulated during the lytic cycle.

It is notable that we have established EBV-positive 293A cell clones with constitutive activation of the lytic cycle, a permissive state resembling EBV infection in OHL (Greenspan et al., 1985). According to our observations, the EBV lytic cycle in 293A lytic clones was maintained at a moderate level below the maximum that could be fully activated (Fig. 6). The moderate level of the lytic cycle in these lytic clones may represent a balanced state of EBV productive infection with minimized cytotoxicity, as the cell viability of lytic clones was similar to that of the latent clones (data not shown). Two possible mechanisms may account for the permissive EBV infection: a rearranged viral genome...
causing constitutive expression of Zta or Rta or a unique cellular factor supporting spontaneous activation of the Zta or Rta promoter (Grogan et al., 1987; Young et al., 1991). No matter which mechanism is the case, the expression of Zta is essentially required as siZ1 suppressed the constitutive EBV activation in 293A lytic clones (Fig. 7). These cell clones are useful for screening and evaluation of agents for inhibiting the EBV lytic cycle. In addition, they also provide good opportunities to explore the mechanisms of spontaneous EBV reactivation and the effects of viral productive infection on epithelial cells.

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