Regulation of Herpesvirus Reactivation by Host MicroRNAs

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The interplay between latent and lytic modes of infection is central to successful infection of all herpesviruses, yet knowledge of the determinants that govern reactivation of these viruses from latent to lytic infection is limited. Recently, several studies have identified roles for specific cellular microRNAs in inhibiting reactivation of various herpesviruses, thereby promoting latent infections. These studies are discussed in the context of current knowledge on mechanisms of regulation of reactivation of specific herpesviruses.

Herpesviruses persist for the life of the host due to the interplay between their latent and lytic modes of infection. In latency, viral gene expression is highly restricted such that few or no viral proteins are expressed, enabling the virus to persist under the radar of the host immune system. The latent virus can then be reactivated to the lytic cycle, which involves an ordered expression of most or all of the encoded proteins, starting with one or two transcriptional activators (referred to as lytic-switch proteins) that initiate the cascade of viral gene expression. In lytic infection, viral genomes are amplified for packaging and virions are produced, enabling cell-to-cell and host-to-host spread. The mechanisms that suppress viral expression in latency and cues that trigger viral reactivation have been the subject of many studies and are only partly understood. The fact that latency tends to occur in specific cell types suggests that the cellular environment is key to determining the infectious cycle of the virus. Several studies have pointed to differences in the chromatin states at viral promoters as common determinants of latent versus lytic infection, and hence, levels of host chromatin-modifying enzymes can impact whether or not a virus reactivates (1, 2). Recently, another common mechanism of promoting herpesvirus latency has emerged: suppression of herpesvirus reactivation by cellular microRNAs (miRNAs).

miRNAs are ~22-nucleotide noncoding RNA molecules that are initially transcribed as long primary RNAs and then are sequentially processed by two RNase III enzymes (Drosha and Dicer) to generate mature miRNAs (3). In complex with an Argonaute protein, the mature miRNA binds through its seed sequence to a complementary sequence mostly in the 3' untranslated region (UTR) of an mRNA, thereby inhibiting its translation or inducing its degradation. miRNAs are encoded both by cellular and viral genomes and can regulate a wide variety of processes. Herpesviruses typically encode multiple miRNAs that are expressed during latent infection. Many studies have indicated that these miRNAs are important for persistent infection, although the precise roles of many of these miRNAs are not yet known (4, 5). In addition, cellular miRNAs are known to impact herpesvirus infections in many different ways, with several recent findings identifying roles in keeping the viruses in a latent state (5).

ALPHAHERPESVIRUSES

The alpha subfamily of herpesviruses establish latency in neurons. Herpes simplex virus type 1 (HSV-1) is the best studied of the alpha family of herpesviruses, and numerous studies have investigated factors contributing to its latency and the steps in its reactivation. It has been known for some time that the noncoding

viral-latency-associated transcript (LAT) is the only gene expressed at high levels in latency and that it promotes latent infection by suppressing lytic-gene expression. LAT can be processed into four miRNAs, one of which inhibits expression of ICP0 through antisense effects (6). ICP0 regulates the latent-to-lytic switch by inducing the degradation of multiple cellular proteins that suppress viral infection, thereby enabling the expression of the lytic genes (7). Recently, Pan et al. (8) asked whether cellular miRNAs specific to neurons might have a role in keeping HSV-1 in a latent state. Their studies focused on miR-138, as it is highly expressed in neuronal cells relative to its expression in other cell types (6, 8, 9). Interestingly, the seed region of miR-138 was found to be complementary to two sites in the 3' UTR of the ICP0 mRNA. In keeping with this finding, an miR-138 mimic was shown to decrease ICP0 mRNA and protein levels in a manner dependent on the target sequences in the 3' UTR. A virus in which the miR-138 target sites in the ICP0 3' UTR were mutated such that they could not be targeted by miR-138 was then generated. The wild-type (WT) and mutant viruses expressed similar levels of ICP0 in Vero cells, but in neuronal cells with abundant miR-138, the expression of ICP0 and other lytic proteins was decreased 2- to 4-fold. Similarly, infection of mouse corneas showed that, compared to WT virus, the mutant virus produced decreased levels of ICP0 and other lytic proteins in the trigeminal ganglia, without affecting the level of the HSV-1 genomes or LAT, and ultimately resulted in increased mortality. The results as a whole identify another level of control of ICP0 expression, and hence of lytic reactivation, through the miR-138 cellular miRNA.

BETAHERPESVIRUSES

Human cytomegalovirus (CMV) is betaherpesvirus that establishes latent infection in monocytes and reactivates to the lytic cycle when the monocytes differentiate to macrophages. Reactivation starts with the expression of two immediate early proteins, IE1 and IE2 (also called UL123 and UL122, respectively), which

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transactivate the expression of all of the lytic genes. IE1 and IE2 are expressed from the major immediate early promoter (MIEP), and therefore regulation of the chromatin structure at MIEP is one way in which viral reactivation appears to be regulated. In addition, O'Connor et al. (10) recently identified cellular miRNAs that target IE2. They predicted that 3 of the 5 hsa-miR-200 miRNA family members (hsa-miR-200b, -200c, and -429) target the 3' UTR of IE2. This prediction was confirmed for hsa-miR-200b by showing that expression of luciferase fused to the 3' UTR from IE2 was downregulated upon overexpression of hsa-miR-200b and that downregulation was dependent on the hsa-miR-200b targeting sequence in the UTR. In addition, overexpression of the C1 hsa-miR200 cluster (hsa-miR200b, -200a, and -429) in fibroblasts was found to decrease IE2 levels in the context of WT viral infection but not during infection by a virus in which the IE2 3' UTR was mutated in the miR-200 target sequence. Moreover, infections of both Kasumi-3 cells (used as a model of latent infection) and human CD34⁺ cells showed that CMV with the IE2 3' UTR region deleted had a higher level of lytic infection than WT virus. Finally, the authors compared the levels of the hsa-miR-200 cluster in undifferentiated cells, where CMV establishes latency, and in differentiated cells that support lytic infection. This cluster was found to be expressed at much higher levels in undifferentiated cells (Kasumi-3 cell line or CD34⁺ or monocyte primary cells) than in differentiated cells (macrophages or Kasumi-3 cells treated with tetradecanoyl phorbol acetate [TPA]). Overall, the results strongly support a model in which the presence of high levels of hsa-miR-200 miRNAs in undifferentiated cells promotes latency by suppressing CMV gene expression and in which the loss of these miRNAs during differentiation promotes lytic infection.

GAMMAHERPESVIRUSES

Epstein-Barr virus (EBV) is a gammaherpesvirus that can undergo latent and lytic forms of infection in both B lymphocytes and epithelial cells. There are multiple forms of EBV latency, characterized by which latency proteins are expressed, and latent infection is known to promote the development of several types of B-cell lymphomas as well as gastric and nasopharyngeal (NPC) carcinomas. Reactivation from latency starts with the expression of the viral BZLF1 protein (also called Zta), followed by BRLF1 (also called Rta), which together transactivate the expression of the lytic genes. Expression of BZLF1 can be suppressed by host ZEB1 and ZEB2 proteins, which bind to specific sequences in the BZLF1 promoter (11, 12). The hsa-miR-200 family members miR-200a, -200b, and -429, target ZEB1 and ZEB2 (13, 14), suggesting that these miRNAs might induce EBV reactivation. In keeping with this prediction, addition of miR-200b or miR-429 to EBV-positive cells expressing ZEB proteins increased EBV reactivation in a manner dependent on the ZEB binding sites, while inhibition of these miRNAs decreased EBV reactivation (15, 16). However, some gastric carcinoma and NPC cell lines do not express ZEB proteins (15) and yet still maintain EBV in a predominantly latent state, indicating that there are additional levels of regulation.

Recently, Mansouri et al. (17) identified the let-7 family of miRNAs as negative regulators of EBV reactivation in gastric carcinoma and NPC cells lines (some of which express ZEB proteins and some of which do not). A screen for miRNAs affected by Epstein-Barr nuclear antigen 1 (EBNA1) expression in NPC cells identified seven let-7 miRNA family members (including let-7a)

as being upregulated by EBNA1. EBNA1 is an EBV latency protein with multiple functions, whose silencing was previously found to promote EBV reactivation in gastric carcinoma cells (18). EBNA1 overexpression increased let-7a levels in multiple cell lines, while EBNA1 depletion decreased let-7a, and these effects appeared to be due to the ability of EBNA1 to transactivate expression of the let-7a primary RNAs. Treatment of EBV-positive gastric carcinoma and NPC cells with a let-7a mimic decreased the percentage of cells that reactivated to the lytic cycle (either spontaneously or in response to TPA and sodium butyrate treatment, which induces EBV reactivation), while treatment with a let-7 sponge had the opposite effect. This suggested that the ability of EBNA1 to promote latent infection may be due to induction of let-7 miRNA. The let-7 target protein that was most consistently changed by let-7a and EBNA1 in these experiments was Dicer, suggesting that high Dicer levels promote EBV reactivation (17). Interestingly, a study by Iizasa et al. (19) also pointed to Dicer as a positive regulator of EBV reactivation, as they showed that Dicer targeting by the EBV miRNA BART6 promotes EBV latency. As part of this study, Dicer depletion was found to decrease the expression of BZLF1 and Rta, consistent with a positive role of Dicer in EBV reactivation. Together, the results indicate that both host let-7 and viral BART6 miRNAs promote EBV latency by downregulating Dicer. Why high Dicer levels induce EBV reactivation remains to be determined.

Like EBV, Kaposi's sarcoma-associated herpesvirus (KSHV) is a gammaherpesvirus that can induce cancer. KSHV reactivation starts with the expression of Rta, a homologue of EBV Rta, which activates the expression of subsequent lytic genes. Two cellular miRNAs have been reported to regulate KSHV reactivation, miR-498 and miR-320d, and both appear to do so by targeting Rta (20). These miRNAs were found to be downregulated by HSV-1 infection, and since HSV-1 infection of cells latently infected with KSHV can induce KSHV reactivation, they were studied as candidates for host miRNA that control the KSHV lytic switch. Reporter assays showed that miR-498 and miR-320d could target the 3' UTR of Rta. In addition, expression of miR-498 and miR-320d mimics in KSHV latently infected cells was found to increase Rta and other lytic protein expression as well as virion production (20). Therefore, these two miRNAs can promote KSHV latent infection and may be part of the mechanism by which HSV-1 superinfection leads to KSHV reactivation.

CONCLUSION

All subfamilies of human herpesviruses have now been found to have their latent-to-lytic switch regulated by one or more cellular miRNAs (summarized in Fig. 1). In most cases, this involves direct targeting of a viral lytic switch protein by the cellular miRNA. The fact that the cellular miRNA and the viral mRNA target have co-evolved to have this arrangement suggests that downregulation of lytic infection is beneficial to both the cell and the virus. For EBV, cellular miRNAs that target the lytic-switch proteins have yet to be reported; rather, the host miRNAs presently known to regulate EBV reactivation target cellular proteins, which in turn impact EBV infection. While the roles of these cellular miRNAs add to our understanding of herpesvirus reactivation, they also raise additional questions about how negative regulation by these cellular miRNAs is overcome to allow lytic infection. This is easy to imagine for viruses, such as CMV, that reactivate in response to cellular differentiation, as the decrease in levels of hsa-miR200 miRNAs

FIG 1 Summary of the regulation of herpesvirus lytic-switch proteins by cellular and viral miRNAs. Viral lytic-switch proteins and some cellular proteins that regulate them are shown in black. Cellular miRNAs are shown in red, and viral miRNAs are shown in blue. Blunted lines indicate negative regulation, and arrows indicate positive regulation.

that accompany differentiation would enable reactivation. However, for regulation of HSV-1 reactivation by miR-138, it remains to be determined how the suppression of ICP0 expression by miR-138 is eventually overcome or whether miR-138 levels are down-regulated in response to specific cues in order to allow viral reactivation in the neuron. No doubt the search for answers to such questions will provide interesting future studies.

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