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Small RNAs growing tall: miRNAs as drug targets in herpesvirus infections

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Herpesviruses establish life-long latent infections. They can cause severe morbidity and significant mortality particularly in immunocompromised hosts. Several are associated with cancers. Most express large amounts of microRNAs during latent or lytic infection. There is increasing evidence that these small RNA molecules play important roles in many aspects of pathogenesis, including lytic and latent infections, immune evasion and tumorigenesis. Therapies targeting microRNAs have already successfully made it into clinics, for example, to treat hepatitis C virus (HCV) infection. In this review, we will focus on regulatory functions of herpesvirus miRNAs that may be suitable for antiviral intervention.

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Introduction

MicroRNAs (miRNAs) are small non-coding RNAs, typically 22 nt in length, which regulate the expression of thousands of genes post-transcriptionally in a wide variety of eukaryotes, from plants and worms to humans [1•]. A long primary transcript (pri-miRNA) is processed into a ≈70–80 nt long precursor miRNA (pre-miRNA) by the RNAse III enzyme Drosha [2,3]. The pre-miRNA is then exported into the cytoplasm, where cleavage by the ribonuclease Dicer generates the mature miRNAs duplex [4,5]. Mature miRNAs bind their mRNA targets as part of the RNA-induced silencing complex (RISC). This results in mRNA degradation and translational repression [6]. Metazoan miRNAs play important roles in a broad variety of biological processes, including development, cell proliferation and differentiation [7]. More than 1000 cellular miRNAs have been identified in humans targeting >50% of known genes. A disruption of miRNA regulation is associated with a broad range of pathologies [8], in particular cancers [9,10].

Herpesviruses are large DNA viruses, which establish life-long latent infections and have co-evolved with their animal and human hosts over millions of years. Interestingly, these viruses independently learned to utilize the cellular miRNA machinery and modulate host and viral gene expression [11–17]. During lytic infection, herpesviruses follow a well-orchestrated program of gene expression comprising hundreds of open reading frames (ORFs) as recently shown for human cytomegalovirus (HCMV) [18••]. During latency, however, transcription of herpesvirus genomes is very limited to avoid recognition by the adaptive immune response [19–21]. The observation that most herpesviruses also express large amounts of viral miRNAs during latent infection suggests an important role of herpesvirus miRNAs during latency and reactivation. In this review, we will focus on conserved and concerted regulation of cellular and viral gene expression by herpesvirus miRNAs to illustrate the challenges and opportunities these small RNA molecules provide for the development of novel antivirals.

Regulation of common cellular pathways and genes by herpesviruses miRNAs

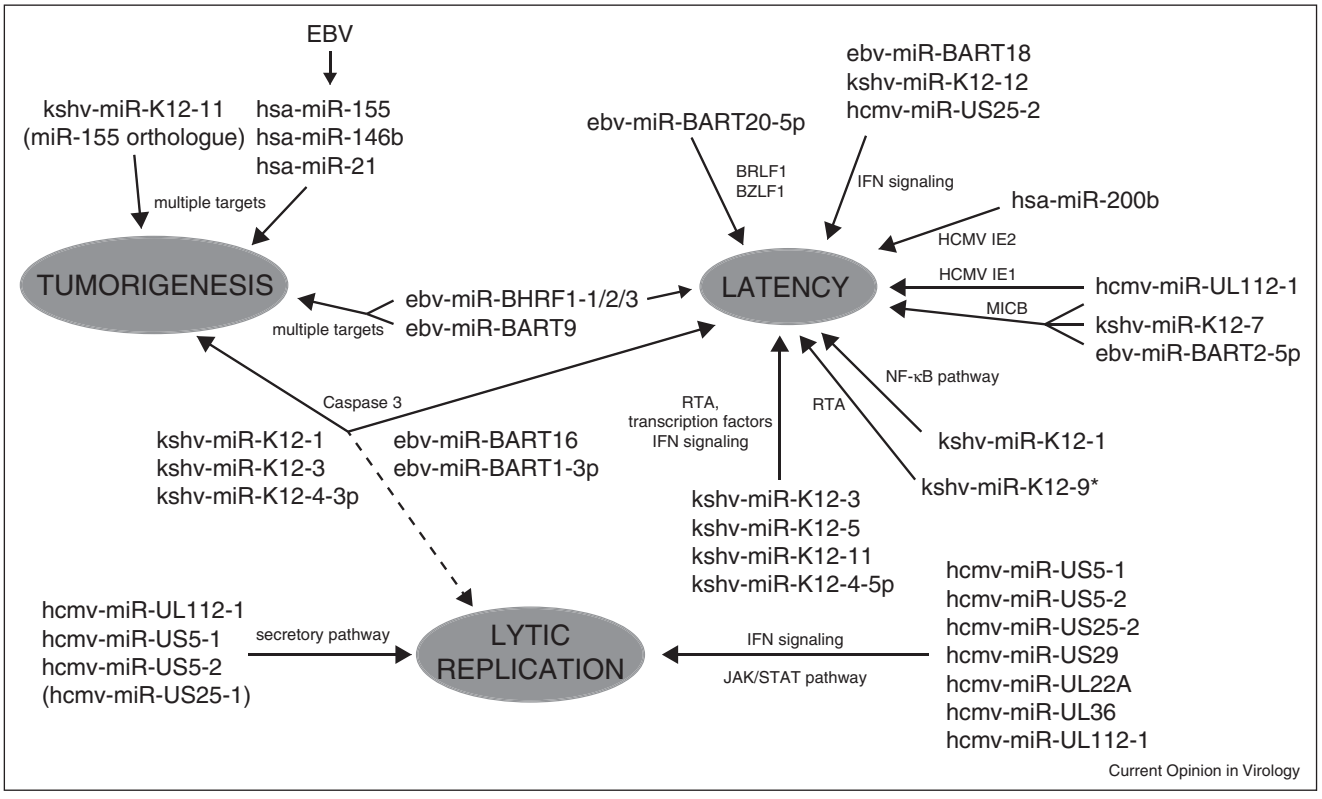
Cellular miRNAs and their target sites show strong evolutionary conservation [22]. In contrast, the evolutionary conservation of herpesvirus miRNAs is rather weak in both genomic position and sequence [23]. Interestingly, recent studies employing high-throughput approaches to identify HCMV, Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) miRNA targets, that is, RISC immunoprecipitation followed by microarray analysis (RIP-Chip) [24,25,26••], high-throughput sequencing of RNA isolated from crosslinking immunoprecipiation (HITS-CLIP) [27,28,29••], photoactivated ribonucleoside-enhanced crosslinking and immunoprecipiation (PAR-CLIP) [30••,31••,32••] and quantitative proteomics [32••,33], demonstrated that their targetomes share the regulation of many genes and pathways. These include the innate immune response, cell cycle, apoptosis, signal transduction and regulation of transcription.

During evolution of cellular miRNAs, families of miRNAs arose by genome duplications, *de novo* birth and hitchhiking, allowing the host cell to expand the regulatory miRNA network without losing important existing features [34,35]. At the same time, their target sites remained remarkably conserved. This resulted in an

extensive, complex network of miRNA-mediated regulation of eukaryotic gene expression. It is hard to imagine that such coordinated regulation is achievable by viral miRNAs. Nevertheless, recent studies highlight an important role of herpesvirus miRNAs in virus pathogenesis. How is this achieved? Data from recent high-throughput studies point at an answer to this question. By expressing sets of up to 45 viral miRNAs at very high levels, cooperative effects between various viral miRNAs gain in importance and are utilized to achieve the desired regulatory effects. As such, it is usually not a single viral miRNA regulating cell cycle, a second one immune evasion and a third establishment of latency. Rather, multiple viral miRNAs expressed from the same genomic locus and/or with similar kinetics cooperate to target genes and pathways important in the virus life cycle. This is best exemplified by a recent report from Hook and colleagues [26••], demonstrating that three HCMV miRNAs (miR-UL112-1, US5-1 ad US5-2) coordinately target the secretory pathway. Their concerted removal from the viral genome had detrimental impacts on productive infection. In addition, ectopic expression of all three miRNAs resulted in the formation of cytoplasmic structures reminiscent of the viral assembly compartment, supporting a key role of these miRNAs in host cell reprogramming during productive infection. Interestingly, another HCMV miRNA targets the endosomal acidification complex component ATP6V0C [25], indicating that the regulatory network of HCMV miRNAs targeting the secretory pathway may well be larger. These findings provide the first evidence for an important role of a subset of viral miRNAs during productive infection, providing a potential target for the development of new antivirals (see Figure 1). However, it is important to note that these HCMV miRNAs also target other genes outside the secretory pathway. For example, miR-UL112-1 targets the viral IE1 gene [36,37], interleukin-32 [38], Toll-like receptor 2 (TLR2) [39] and the antiviral restriction factor BCLAF1 [40].

Another example of concerted regulation is the targeting of Caspase 3 by three KSHV miRNAs [41] and two EBV miRNAs [42••]. Only the combined knockdown of all three KSHV miRNAs increased the cell's susceptibility to apoptosis. Whether this is solely due to the lack of Caspase 3 regulation or involves targeting of other pro-apoptotic genes remains to be elucidated. In addition, single miRNAs of EBV, KSHV and HCMV all target the stress-induced NK cell activating ligand MICB [43,44],

Figure 1



Viral and cellular miRNAs as therapeutic targets in herpesvirus infections Schematic representation of candidate miRNAs for antiviral intervention (EBV, KSHV and HCMV). Known targets of miRNAs are indicated next to the arrows. The dashed arrow indicates a potential role for regulation of Caspase 3 in KSHV lytic replication.