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Review

The role of microRNAs in Epstein-Barr virus latency and lytic reactivation

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Abstract

Oncogenic viruses reprogram host gene expression driving proliferation, ensuring survival, and evading the immune response. The recent appreciation of microRNAs (miRNAs) as small non-coding RNAs that broadly regulate gene expression has provided new insight into this complex scheme of host control. This review highlights the role of viral and cellular miRNAs during the latent and lytic phases of the EBV life cycle.

Keywords: Epstein-Barr virus, EBV; microRNA, miRNA; B-cell lymphoma; LMP1; miR-155; miR-34; miR-146; miR-200; Lytic reactivation; ZEB

1. Introduction

MicroRNAs (miRNAs) are small, ~21–25 nucleotide, non-coding RNAs expressed by all multicellular eukaryotes that negatively regulate gene expression by targeting complementary sequences in messenger RNAs [1]. These regulatory RNAs have been demonstrated to play a key role in a variety of processes including development, cell cycle regulation, and immunity and their malfunction has been associated with several human pathologies including cancer [2]. MiRNAs perform their gene regulatory function as the guide RNA component of the RNA-induced silencing complex (RISC) complex, which binds perfect or partially complementary sequences predominantly found in the 3′UTR of target mRNAs, causing mRNA translation inhibition or mRNA degradation. As miRNAs require only limited complementarity for mRNA binding, they are able to modulate the expression of multiple genes. Conversely, different miRNAs can control a single mRNA, making miRNA regulatory networks particularly complex to investigate. The region that dictates the specificity of the miRNA:mRNA target interaction corresponds to nt 2–8 from the miRNA 5′ end and is referred to as the "seed" sequence. Seed sequences can be shared by several distinct miRNAs, which are termed members of the same seed family [3].

MiRNAs are generally produced as RNA polymerase II-driven, capped, and poly-adenylated RNA precursors [1]. Stem-loop structures within these primary miRNAs (pri-miRNAs) are recognized by the enzyme Drosha and processed to yield ~65–70 nucleotide precursor miRNAs (pre-miRNAs), which are subsequently exported from the nucleus to the cytoplasm through an Exportin 5-dependent pathway. The pre-miRNA is then recognized by a complex containing the RNAse III enzyme Dicer, which liberates a duplex intermediate of ~22 base pairs. One strand of this duplex is then loaded into the RISC composed of Argonaute family proteins and accessories. The mature miRNA guides the RISC complex to target mRNAs through its seed sequence to enable suppression of target expression.

The identification and characterization of cellular as well as virally-encoded miRNAs have established their roles as broad and important regulators of the host/pathogen interface [4]. The major family of viruses that encode and modulate miRNAs is the Herpesviridae. These large double-stranded

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DNA viruses typically contain nearly one hundred protein coding genes and it is now evident that many miRNAs are also encoded in their genomes. In particular, the oncogenic γ-herpesviruses encode a large number of miRNAs and also modulate host miRNAs as a means of effecting cell transformation. An important human pathogen and model system for studying the role of miRNAs in viral oncogenesis is the γ-herpesvirus Epstein-Barr virus (EBV).

EBV infects greater than 90% of adults worldwide [5]. Despite the high rate of prevalence, disease is rarely manifested in infected individuals due to a strong cytotoxic T cell response. In immune-compromised individuals, such as those infected with HIV or following transplant, EBV-associated malignancies are more common. Furthermore, EBV is causally implicated in African endemic Burkitt's lymphoma (BL) and the epithelial cancer nasopharyngeal carcinoma (NPC). Acute infection during adolescence also leads to infectious mononucleosis due to the uncontrolled expansion of polyreactive B cells.

EBV is a large, enveloped virus containing a ~184 kbp double-stranded DNA genome. *In vivo*, B lymphocytes and epithelial cells are common targets, while rare infection of NK and T cells has also been observed [5]. Infection of primary B cells *in vitro* leads to a latent infection in which only a subset of viral genes are expressed including the latent membrane proteins 1, 2A, and 2B, Epstein-Barr nuclear antigens (EBNAs) 1, 2, 3A, 3B, 3C, and LP, the small non-coding EBER RNAs, as well as 25 viral pre-miRNAs. This expression program is called latency III and drives the indefinite proliferation of primary B cells (Table 1). In other settings *in vivo* including BL tumors, Hodgkin's lymphoma (HL), and NPC, EBV displays a more restricted form of latency (Table 1). Finally, in normal infected individuals, the virus exists in memory B cells in the peripheral blood where no genes are expressed except for EBNA1 during cell division [6,7]. Studies of EBV-infected B cells and tumor-derived cell lines have informed much of our understanding of the mechanisms by which EBV drives tumorigenesis [5].

Infection of either B lymphocytes or epithelial cells with EBV poses several barriers to long-term persistence in the host. Both the innate and adaptive immune response can prevent virus replication and the growth of virus-infected cells. Therefore, the virus ensures control of host physiology by regulating host cell gene expression. This occurs both through modulation of specific signaling pathways as well as by restricting its own gene expression. For example, LMP1 mimics a constitutively active CD40 (B-cell co-stimulatory TNFR family member) [8], while LMP2A mimics the B-cell receptor (BCR) and antagonizes endogenous BCR signaling [9]. Fundamentally, restriction of viral gene expression, for example in latency I, prevents CD8+ T cell recognition of immune-dominant epitopes in the EBNA3 proteins, and enables long-term persistence of latently-infected cells. Lastly, EBV latent infection also depends on tight control of the viral lytic transactivator protein Zta.

The primary effects of EBV on host cell physiology are mediated through changes in host gene expression. Given the importance of miRNAs in regulating gene expression, many studies have now implicated miRNAs in mechanisms through which EBV modulates the host. These reports will be highlighted in this review covering five major areas: i) the expression of EBV-encoded miRNAs, ii) mRNA targets and functional significance of EBV miRNAs, iii) the regulation of cellular miRNA expression during EBV infection, iv) the functional role of cellular miRNAs in EBV latency and lytic reactivation, and v) genome-wide methods to identify mRNA targets of miRNAs in EBV-infected cells.

2. Expression of Epstein-Barr virus encoded miRNAs

2.1. EBV miRNA expression in infected cells and tumors

EBV was the first human virus shown to express miRNAs and to date is the virus that encodes more miRNAs than any other human virus, with twenty-five identified pre-miRNAs. Pfeffer et al. were the first to show that EBV expresses miRNAs by cloning small RNAs from an EBV-infected Burkitt's lymphoma cell line [10]. In this study, 5 viral miRNAs, located in two distinct clusters were identified. One cluster is located near the mRNA of the BHRF1 (BamHI fragment H rightward open reading frame 1) gene, coding miR-BHRF1-1 to 3, while the other is located in intronic regions of the BART (Bam HI fragment A rightward transcript) giving origin to miR-BART1 and 2. Since this initial report, other groups have identified additional EBV miRNAs, all of them located within the BART cluster. Cai and

Table 1
EBV latency gene expression programs.

	Latency I	Latency II	Wp-Restricted	Latency III
Viral protein expression	EBNA1	EBNA1, LMP1, LMP2A, 2B	EBNA1, 3A, 3B, 3C, LP LMP1, 2A, 2B BHRF1	EBNA1, 2, 3A, 3B, 3C, LP LMP1, 2A, 2B
EBERs	Yes	Yes	Yes	Yes
miRNAs	BART miRNAs (modest)	BART miRNAs (high)	BHRF1 miRNAs (modest) BART miRNAs (modest)	BHRF1 miRNAs (high) BART miRNAs (modest)
Diseases/ cell states	Burkitt's lymphoma	Nasopharyngeal carcinoma, Hodgkin's lymphoma	Burkitt's lymphoma	Post-transplant Lymphoproliferative Disease, HIV lymphomas, Diffuse large B cell lymphomas, Lymphoblastoid cell lines