microRNAs of Epstein-Barr virus control innate and adaptive anti-viral immunity

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Abstract

More than 90% of humanity is infected with Epstein-Barr virus (EBV) lifelong. While infection is usually controlled by the immune system, the human host fails to completely eliminate the pathogen. Several herpesviral proteins are known that act as immunoevasins preventing or reducing recognition of EBV-infected cells. Only recently, microRNAs of EBV were identified to reduce immune recognition further. This Gem summarizes what we know about immunomodulatory miRNAs of herpesviruses.

Keywords

Immune surveillance, immune evasion, herpesvirus, microRNA, cancer

Introduction

Epstein-Barr Virus (EBV) is a successful human herpesvirus that infects about 90% of the human population. Upon infection, EBV reprograms resting, quiescent B lymphocytes, the main reservoir of this virus, to become activated, antigen-presenting cells, which are detected by various immune cells and become targets of the host's immune surveillance. EBV nonetheless can establish a latent, life-long infection in the memory B cell compartment, in part, through its virally encoded immunoevasins, i.e. proteins that fend off both the innate and adaptive immune responses of its human host (1).

MicroRNAs (miRNAs) are small regulatory RNAs of 19-22 nucleotides in length. They usually bind to 3'UTRs of targeted mRNAs affecting their stability. Down-regulation is often modest (<50%) and miRNAs are thought, therefore, to 'fine-tune' gene expression (2). Single miRNAs can potentially target hundreds of different mRNAs because the minimal requirement to bind the target mRNAs is the 6 nt-long "seed" sequence. Similarly, single mRNAs can be bound and regulated by multiple miRNAs forming a complicated regulatory network. Human miRNAs are involved in a wide range of physiological functions such as development, growth, differentiation, apoptosis, stress response, and immune regulation (2, 3).

EBV was the first virus found to encode miRNAs (4, 5) and is the largest reservoir of miRNAs among human herpesviruses known to date. EBV encodes at least 44 miRNAs, which can potentially regulate hundreds of genes, but their identity is just beginning to emerge. EBV's miRNAs are expressed in all phases of its complicated life cycle as well as in EBV-associated tumors (6). Accumulating evidence so far has shown that EBV's miRNAs promote survival and proliferation of infected B cells early during infection as well as in tumor cells (7–10), but viral miRNAs have also been found to modulate immune evasion (11, 12). Viral miRNAs in different herpesviruses are rarely conserved (13), but some regulate the same targets (14, 15).

In this brief review, we focus on miRNAs of EBV and other human herpesviruses and their recently identified functions in regulating innate and adaptive immune responses, which lead to

these viruses' immune evasion (Fig 1). We shall also discuss the potential roles of circulating viral miRNAs and their possible implications in clinical practice.

Viral miRNAs and innate immunity

Innate immune responses form the first line of defense against infectious agents; accordingly, viral miRNAs target several cellular transcripts in this pathway to escape immediate detection (Fig 1). Type-I interferons (IFN), secreted in response to viral infection, play a central role in antiviral immunity and activate STAT transcription factors, which in turn induce the transcription of IFN-stimulated genes (ISG) in infected and neighboring cells leading to multiple, antiviral functions. Diverse herpesviral miRNAs target components of the Type-I IFN signaling pathway including STATs (16, 17) limiting the antiviral effects of ISGs.

Another important arm of innate immunity are natural killer (NK) cells, which sense different activating and inhibitory molecules on the surface of stressed or virally infected cells. When induced upon stress the MICB surface molecule activates NK cells by binding to its receptor NKG2D (18). Several miRNAs of human herpesviruses including EBV, Kaposi-sacroma associated herpesvirus (KSHV), and human cytomegalovirus (HCMV) have been reported to control the *MICB* transcript reducing NK cell recognition and killing of virally infected cells (14, 19).

The regulation of inflammation is a common goal of viral miRNAs. Cytokine synthesis is regulated by EBV miRNAs upon infectious stimuli in nasopharyngeal cancers (NPC) (20) as well as by KSHV miRNAs in lymphomas (21). Human miR-155 can have positive and negative consequences for the immune response (22), but miR-155 negatively regulates Toll-like receptor signaling pathways presumably reducing the pro-inflammatory responses to an invading pathogen (3). Interestingly, KSHV miR-K12-11 mimics this human miRNA (15) and EBV also induces miR-155, because the latent membrane protein 1 (LMP1) of EBV activates its expression (23). Conversely, LMP1 itself is a direct target of several EBV miRNAs (24, 25) suggesting that they reduce or limit LMP1 signaling and may thus fine-tune innate immune responses directed against EBV.

Viral miRNAs and adaptive immunity

Among the main components of the adaptive immune response are T cells and antigen-presenting cells (APCs). Antigen presentation of viral peptides by APCs, i.e. EBV-infected B cells, is a multistep process and viral EBV's miRNAs interfere with these steps to reduce the immunogenicity of infected cells. Cytokines and chemokines enhance adaptive immune responses and herpesviruses appear to use their miRNAs to reduce the inflammatory microenvironment of infected cells as well (Fig 1).

Levels of viral antigens. Controlling the abundant expression of viral genes can be a strategy of viral miRNAs thus limiting levels of viral antigen. As a first example, SV40-encoded miRNAs were found to reduce SV40's T antigen expression, protecting infected cells from T cell recognition (26). Similarly, EBV's miRNAs have been reported to target several viral genes downregulating them. Viral miRNAs limit the expression of the EBV proteins EBNA1 (25), LMP1 (27), and LMP2A/B (28) in infected B cells early after infection (Fig 2). EBNA1 is required to maintain the viral genome in infected cells and to distribute genomes equally to daughter cells in mitosis. This protein is commonly expressed during latent infection and has the intrinsic ability to prevent processing and presentation of its epitopes on MHC class I molecules (29). Nevertheless, EBNA1 is a target of effector T cells, but viral miRNAs also limit EBNA1´s immunogenicity reducing its protein levels (25). These findings suggest that viral miRNAs can act as immunoevasins not only in lytic but also in latent infection.

Antigen processing and presentation. Recently, herpesvirus miRNAs were found to regulate cellular genes involved in antigen processing and presentation. HCMV miRNA miR-US4-1 was reported to control MHC class I antigen presentation by targeting ERAP1 (30), but this finding is controversial (31). ERAP1 is an aminopeptidase that optimizes peptide-MHC class I binding and its downregulation leads to a reduced killing of infected cells by virus-specific T cells (30). We reported that EBV miRNAs also regulate antigen processing and epitope transport in infected primary human

B cells (25, 27) down-regulating the TAP complex and lysosomal enzymes affecting MHC class I and class II-mediated epitope presentation, respectively (Fig 2).

EBV miRNAs not only reduce the processing but interfere with the presentation of viral antigens. We observed that cell surface MHCs and co-stimulatory molecules, necessary components for effective antigen presentation, are decreased by viral miRNAs. MHC and co-stimulatory molecules do not seem to be direct targets of viral miRNAs (25, 27) (Fig 2), but these surface molecules might be under control of LMP1, which is limited by several EBV miRNAs. LMP1 mimics CD40 signals in B cells and thus induces MHCs and co-stimulatory molecules, LMP1's down-regulation by viral miRNAs will reduce the immunogenicity of EBV-infected cells. It thus appears that several viral miRNAs balance or even diminish LMP1 expression during latent infection in B cells.

Controlling chemokines and cytokines. Several chemokines and cytokines which regulate antiviral inflammatory responses are targets of multiple viral miRNAs. EBV's miR-BHRF1-3 targets *CXCL11* (32), a chemoattractant of T cells, and thus may reduce local inflammation and T cell recruitment (Fig 2). After EBV infection, primary B cells secrete various inflammatory cytokines including IL-6 and IL-12. We found reduced levels of these cytokines in B cells expressing EBV's miRNAs early in infection. At least five viral miRNAs directly target IL-12p40, hence reducing the secretion of IL-12 and IL-23, both being members of the IL-12 family, from infected B cells. IL-12's best known function is promoting the differentiation of naive $CD4^+$ T cells to anti-viral Th1 cells, a function inhibited by EBV's miRNAs (27) (Fig 2).

In the lytic phase during *de novo* virus synthesis multiple viral proteins are expressed that act as immunoevasins to protect the cells from virus-specific effector T cells. During the early days of infection in the pre-latent phase, comparatively few viral immunomodulatory proteins are expressed (33). During this early phase and presumably also during latency, EBV uses its many non-immunogenic miRNAs as alternative immunoevasins to protect the virus-infected cells from adaptive immune responses.

Circulating viral miRNAs

Extracellular, circulating miRNAs in the bloodstream are considered as potential biomarkers as a result of their disease-specific expression patterns. Circulating miRNAs of EBV have been proposed to serve as diagnostic markers in patients with nasopharyngeal carcinoma, for example (34).

The biological functions of circulating viral miRNAs are currently under investigation. EBV miRNAs contained within extracellular vesicles (EVs) were reported to be released constantly from lymphoblastoid B cell lines (35, 36). EVs can be taken up by different cell types, including monocytes and monocyte-derived dendritic cells (35), plasmacytoid dendritic cells (37), and epithelial cells, mainly via caveola-dependent endocytosis (38). Transfer of viral miRNAs to cells can lead to the repression of target genes (35); for example, miR-BART15 represses the inflammasome protein NLRP3 in a monocytic cell line (39). The putative functions of EV-contained miRNAs are controversial, because their abundance in EVs is low (40). Interestingly, infectious EBV particles also contain miRNAs (41) suggesting that they can be passively delivered, i.e. transduced during infection to exert so far unknown but, perhaps, immunoevasive functions in recipient cells.

Outlook

EBV expresses viral miRNAs in the pre-latent phase immediately after infection, during latency, and in the lytic, productive phase. Particularly in the pre-latent and latent phases, when the expression of viral immune evasion proteins is limited, the many viral miRNAs are likely to have important immunoevasive functions. Viral miRNAs are non-immunogenic and are transcribed and processed like miRNAs of the cellular host. EBV and other herpesviruses (except beta herpesviruses such as cytomegalovirus) encode miRNAs in gene clusters insuring their simultaneous expression and potential, co-operative functioning. The IL-12 and the STAT1 signalling pathways, which are targeted by multiple miRNAs encoded by EBV or KSHV, respectively, are revealing examples of the

potential of viral miRNAs to repress single genes or pathways, a function that goes well beyond fine-tuning of single genes.

In this light, it is remarkable to learn that miRNAs of different members of the herpesvirus family are distinct, sharing little sequence conservation, with few exceptions (15, 42). Lack of sequence conversation imposes a general difficulty for researchers because conservation across species is one of the best parameters to predict the targets of miRNAs. Instead, each herpesvirus has evolved its own set of miRNAs probably to adapt to the RNA synthetic networks in the different cell types human herpesviruses infect. miRNAs of different herpesviruses rarely target the identical transcripts in the different host cells but rather alter the same global functions such as anti-viral immunity.

During infection, herpesviruses use their miRNAs to evade immune surveillance by the host. EBV-associated tumor cells also express viral miRNAs (6), and it seems plausible that they also reduce the anti-tumor response of infiltrating immune cells. Several strategies have been developed recently to block miRNA functions directed at indications other than EBV-associated diseases and some have reached the phase of clinical studies (43). If successful, similar blocking of viral miRNAs in EBV-associated tumors may restore functional anti-tumor immunity, and thereby benefit patients with these tumors.

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Figure legends

FIG 1 Immunoevasive functions of herpesviral miRNAs. miRNAs of the human herpesviruses EBV, KSHV, and CMV target cellular and viral genes regulating the anti-viral responses of innate and adaptive immunity. Shown are key genes down-regulated by viral miRNAs.

FIG 2 EBV's miRNAs globally control anti-viral adaptive immune responses in infected cells. Upon infection, the viral DNA genome circularizes and viral coding and non-coding RNAs are expressed immediately. EBV miRNAs support the evasion of adaptive immunity at several levels: (1) viral miRNAs down-regulate viral transcripts to limit viral antigen synthesis: miR-BART22 controls LMP2A/B, several BART miRNAs control LMP1, and EBNA1 is controlled by unidentified viral miRNAs. (2) Reduced levels of LMP1 may lead to lower levels of antigen presentation, because LMP1 activates co-receptors and MHCs expression. (3) Viral miRNAs control antigen processing for MHC class I-mediated presentation regulating the expression of TAP2, a target of miR-BHRF1-3 and -BART17. (4) miR-BART1 and -BART2 control the expression of the lysosomal enzymes IFI30 and LGMN, respectively; a third lysosomal enzyme, CTSB, is controlled by both miR-BART2 and -BHRF1-2, reducing the capacity to present antigenic epitopes on MHC class II molecules to $CD4^+$ T cells. (5) Secretion of the NK cell ligand CXCL-11 is reduced by miR-BHRF1-3 while the mRNA encoding inflammatory cytokine IL-12 (and two additional cytokines, IL-12B and IL-23) is directly bound by five EBV miRNAs resulting in suppressed Th1 differentiation. Other inflammatory cytokines such as IL-6 are also reduced by viral miRNAs.