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The roles of EBV-encoded microRNAs in EBV-associated tumors

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ABSTRACT

Epstein-Barr virus (EBV) is believed to be a pathogen causing a number of human cancers, but the pathogenic mechanisms remain unclear. An increasing number of studies have indicated that EBV-encoded microRNAs (EBV miRNAs) are expressed in a latency type- and tumor type-dependent manner, playing important roles in the development and progression of EBV-associated tumors. By targeting one or more genes of the virus and the host, EBV miRNAs are responsible for the deregulation of a variety of viral and host cell biological processes, including viral replication, latency maintenance, immune evasion, cell apoptosis and metabolism, and tumor proliferation and metastasis. In addition, some EBV miRNAs can be used as excellent diagnostic, prognostic and treatment efficacy predictive biomarkers for EBV-associated tumors. More importantly, EBV miRNA-targeting therapeutics have emerged and have been developing rapidly, which may open a new era in the treatment of EBV-associated tumors in the near future.

1. Introduction

Epstein-Barr virus (EBV), a ubiquitous γ-herpesvirus, establishes latent infection in almost all human beings. It was the first virus identified to be associated with human cancer (Young and Rickinson, 2004). Almost all cases of nasal NK/T-cell lymphoma, undifferentiated nasopharyngeal carcinoma (NPC) and lymphoepithelioma-like carcinomas (LELCs), and approximately 95% of endemic Burkitt's lymphoma (BL), 90% of post-transplant lymphoproliferative disorder (PTLD), 50–80% of Hodgkin's lymphoma (HL), 15–30% of sporadic BL, 15% of diffuse large B-cell lymphoma (DLBCL), about 10% of gastric carcinoma (GC) have been found to be closely linked with EBV infection (Delecluse et al., 2007; Lung et al., 2013).

During latent infection, only a limited number of EBV genes are expressed, including those that encode six EBV nuclear antigen (EBNA) proteins (EBNA-1, EBNA-2, EBNA-3 A, 3B, 3C and EBNA-LP), three latent membrane proteins (LMP1, LMP2A and LMP2B), and EBV-encoded RNAs (EBER-1 and EBER-2). There are four latency types according to the selective expression of viral proteins (Young and Rickinson, 2004). Latency 0 is only observed in healthy carriers, in which no viral proteins are produced or only low levels of EBNA-1 and LMP2A can be detected in B lymphocytes. BL is a representative of latency I, and viral protein expression is limited to EBNA-1 in this type. Latency II is characterized by expression of EBNA-1, LMP1, LMP2A and LMP2B, and is the state commonly found in NPC, HL and NK/T-cell lymphoma. In latency III, often seen in immunodeficiency associated lymphoma, such as PTLD and acquired immune deficiency syndrome (AIDS)-related lymphoma, all six EBNAs and three LMPs are expressed. It is noteworthy that although EBNA-1 is expressed and LMP1 and LMP2B are absent in EBV-associated GC (EBVaGC), LMP2A is also present in about half of EBVaGC cases (Sugiura et al., 1996; Luo et al., 2005). Therefore, EBVaGC may have a latency pattern between type I and type II.

miRNAs are 18–24 nucleotide non-coding RNA molecules. By inhibiting translation or degrading target mRNA, miRNA serves as an indispensable part in the network of gene regulation. EBV was the first virus found to encode miRNAs (Pfeffer, 2004). To date, 25 miRNA precursors with 44 mature miRNAs produced by EBV have been identified. As shown in Fig. 1, 4 miRNAs are encoded by the *Bam*HI fragment H rightward open reading frame 1 (BHRF1) region, and the remaining 40 miRNAs are generated from the *Bam*HI A rightward transcript (BART) region. EBV miRNAs have been suggested to play important roles in latency maintenance, immune evasion and development and progression of EBV-associated tumors by regulating both viral and human gene expression. In this review, we will discuss the expression, function and clinical significance of EBV miRNA in EBV-associated tumors.

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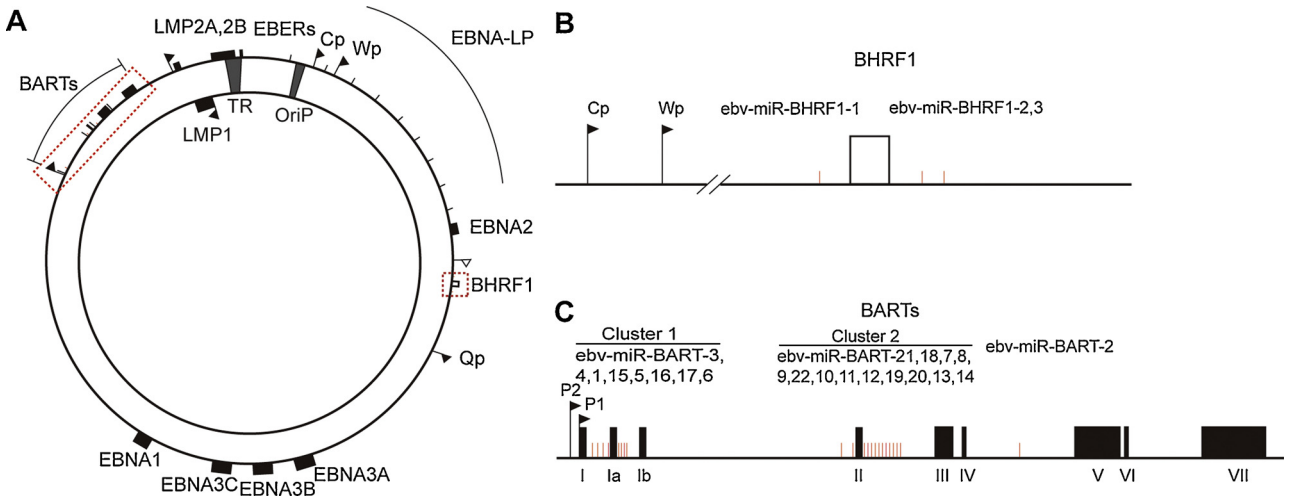

Fig. 1. Location of BHRF1 and BART miRNAs on the EBV genome. (A) Position of latent genes on the double-stranded EBV genome. (B) Position of BHRF1 miRNAs on a linear genome. (C) Position of BART miRNAs on a linear genome. The promoters and exons of *BHRF1* are indicated as white pennants and boxes, respectively. The black pennants and boxes represent promoters and exons of *BART*, respectively. The exons of *BART* are labeled with Roman numerals. The location of EBV miRNAs is indicated by red lines (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2. Expression of EBV miRNAs in EBV-associated tumors

2.1. EBV miRNA expression in lymphoma

Most of the miRNAs encoded by EBV were initially discovered in lymphoma cell lines. Pfeffer et al. (Pfeffer, 2004) first identified five EBV miRNAs (BHRF1-1, -2, -3, BART1 and 2) in the BL41/95 cell line using molecular cloning methods. Subsequently, with bioinformatics and microarray-based technologies, Grundhoff and colleagues discovered an additional 18 BART miRNAs (BART3 to BART20) in the Jijoye BL cell line (Grundhoff et al., 2006). In recent years, development of next-generation sequencing has made it easier to identify new miRNAs and profile miRNA expression. In EBV-positive DLBCL samples, EBV miRNAs detected by next-generation sequencing represented 1.7% of all miRNAs, in which all known BART miRNAs were present with the exception of BART15 and BART22. In this profile, BART7, BART22, BART10, BART11-5p and BART16 were the most highly expressed BART miRNAs (Imig et al., 2011). In NK/T-cell lymphoma samples, EBV miRNAs accounted for 2.3% of the total miRNAs. The five most highly expressed EBV miRNAs in NK/T-cell lymphoma were BART7, BART5, BART11-5p, BART1-5p and BART19-3p (Motsch et al., 2012). A recent study demonstrated that 2.7% of the miRNAs detected in endemic BL samples were EBV miRNAs, in which BART7, BART10, BART11-3p, BART6-3p and BART17-5p were the most highly expressed BART miRNAs (Oduor et al., 2017). However, the expression levels of BART miRNAs in HL were much lower than in other tumor types, and this might due to the low number of tumor cells in HL samples (Qiu et al., 2011; Sakamoto et al., 2017). Of note, BHRF1 miRNAs were not detected in all these types of lymphoma but were detected in PTLD and AIDS-related DLBCL, in which high levels of both BHRF1 and BART miRNAs were expressed (Fink et al., 2014; Xia et al., 2008).

2.2. EBV miRNA expression in epithelial malignancies

Cosmopoulos and colleagues profiled the EBV miRNA expression using multiplex reverse transcription-PCR and found that the most abundant EBV miRNAs in NPC biopsy samples were BART17-3p, BART7, BART22, BART9, and BART14* (Cosmopoulos et al., 2008). Deep sequencing in NPC samples showed that EBV miRNAs comprised 23.3% of the total miRNAs, with BART3-3p, BART9-3p, BART5-5p, BART4-5p, and BART7-3p being the most highly expressed (Chen et al., 2010). In a miRNA microarray-based EBV miRNA profile in NPC samples, BART7, BART1-3p, BART8*, BART3 and BART5 have high abundance (Wong et al., 2012). Another study demonstrated that EBV miRNAs accounted for 5% and 19% of total miRNAs in two NPC samples, respectively, and the most abundant EBV miRNAs were BART1, BART4, BART6, BART7, BART11(Zhu et al., 2009).

Kim and colleagues first described the expression of EBV miRNAs in EBVaGC and found that BART1, BART5, BART7, BART10 and BART12 could be easily detected by Northern blotting in both EBVaGC tissues and cell lines (Kim et al., 2006). A study profiling EBV miRNAs expression with quantitative reverse transcriptional polymerase chain reaction (RT-PCR) revealed that the most abundant EBV miRNAs in EBVaGC samples and cell lines were BART7-3p, BART1-3p, BART9-3p, BART5-5p, and BART10-3p (Shinozaki-Ushiku et al., 2015). Another study revealed that BART4-5p, BART11-3p, BART2-5p, BART6-3p and BART9-3p were the most highly expressed EBV miRNAs in EBVaGC samples using stem-loop quantitative PCR, and the expression pattern of EBV miRNAs in EBVaGC cell line SNU719 was similar to that in tumor tissues (Tsai et al., 2017). Expression of EBV miRNAs in EBV-infected AGS GC cell lines was also profiled by deep sequencing. In this profile, about 15% of total miRNAs were EBV-encoded miRNAs, and BART8-5p, BART10-3p, BART7-5p, BART6-3p and BART22 were the most abundant (Marquitz et al., 2014).

LELC is a rare type of carcinoma which can derive from multiple anatomical sites. The latency type of EBV in LELC is unclear, and few studies have focused on the EBV miRNAs expressed in LELC. It is reported that the BART miRNA profiles in LELC from lung and lymphoepithelioma-like cholangiocarcinoma are similar to those in NPC and EBVaGC (Lung et al., 2013).

BHRF1 miRNAs are absent in all these EBV miRNA profiles from epithelial malignancies.

2.3. Regulation of EBV miRNA expression

Profiling of EBV miRNAs in EBV-infected normal and neoplastic tissues showed that EBV miRNAs are expressed in a latency specific pattern (Qiu et al., 2011). To date, BHRF1 miRNAs are only detected in some in vitro-transformed lymphoblastoid cell lines (B95-8, Jijoye and Namalwa, etc.) (Cai et al., 2006; Kim and Lee, 2012) and specific types of lymphoma in immunodeficient patients (Fink et al., 2014; Xia et al., 2008), all of which adopt latency III pattern of EBV gene expression. Latency I and II tumors and cell lines do not express BHRF1 miRNAs, suggesting BHRF1 miRNA expression is restricted to EBV latency type

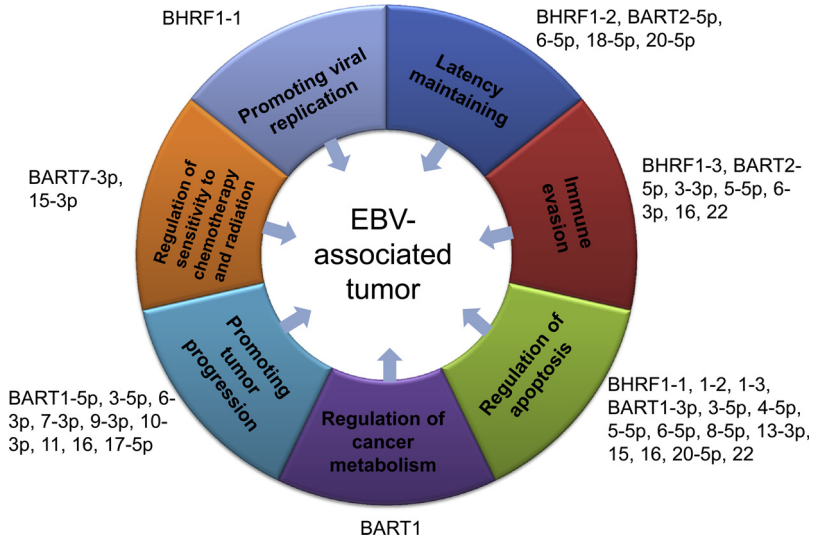

Fig. 2. Functions of EBV miRNAs in the development and progression of EBV-associated tumors. EBV miRNAs contribute tumor development and progression by targeting both viral and host cell transcripts.

III. A number of studies have suggested that promoter methylation might explain the latency type-specific expression of BHRF1 miRNA. Transcription of BHRF1 miRNA depends on the activity of the *Bam*HI C promoter (Cp) and *Bam*HI W promoter (Wp). In latency III cell lines, the Cp and Wp promoters are active, leading to high expression levels of BHRF1 miRNAs (Cai et al., 2006). However, in type I or II latency, CpG residues at Cp and Wp are methylated and BHRF1 miRNAs are not transcribed (Tao and Robertson, 2003; Li and Minarovits, 2003).

Although BART miRNAs are expressed in all the three latency types (latency I-III), their abundance also greatly differs in different EBV-associated tumors and cell lines. According to next-generation sequencing data, the expression levels of BART miRNA in NPC and EBVaGC (about 20% and 15% of total miRNAs, respectively) (Chen et al., 2010; Marquitz et al., 2014) are much higher than those in lymphoma (approximately 2%) (Imig et al., 2011; Motsch et al., 2012; Oduor et al., 2017). In addition, there are substantial differences in abundance of the individual BART miRNAs within a cell line, with levels of the most and least abundant BART miRNAs differing by 50-fold or more, and expression levels of BART miRNAs among cell lines can also differ nearly 50-fold (Pratt et al., 2009). Studies have indicated that promoter methylation, transcription factors, and miRNA processing may contribute to the differential expression of BART miRNAs.

Demethylation of the CpGs within the promoters of *BART* genes may explain the upregulation of BART miRNA expression. Cell lines with hypermethylated *BART* promoters (Mutu 1, Namalwa) have low BART miRNA expression, while those with hypomethylated *BART* promoters (LCL1, SNU-1103 and SNU-20 for lymphoma, C15 for NPC) display higher expression of BART miRNAs (Kim et al., 2011).

Another component of BART miRNA regulation is transcription factors. The P1 promoter, which is a main promoter for BART miRNA expression in NPC and Akata cell line (Kim et al., 2011), can be negatively regulated by interferon regulatory factor (IRF) family members, including IRF5 and IRF7. Interestingly, IRF5 itself is more highly expressed in B cells than in epithelial cells, and IRF7 is absent from NPC tumors (Chen et al., 2005). In addition, the binding sites of other transcription factors such as specificity protein 1 (SP1) and activator protein 1 (AP-1) were also found in the P1 promoter region (de Jesus et al., 2003). The P2 promoter, which is highly activated in epithelial cell lines, can be positively modulated by c-myc and CCAAT/enhancer binding protein (C/EBP) family members (Chen et al., 2005). C-myc is upregulated due to chromosomal translocation in BL (Klein, 1983) and by LMP1 in EBV infected epithelial cells (Chen et al., 2003). C/EBP proteins are expressed at low levels in B-cell lines and BL cells, but at significantly higher levels in epithelial cell lines (Bundy and Sealy, 2003). In addition, there are binding sites for NF-κB in both P1 and P2 regions. Expression of BART miRNAs are upregulated by NF-κB, which can be activated by LMP1, a heterogeneously expressed protein in NPC tumors (Verhoeven et al., 2016). Differential levels of these transcription factors may contribute to the differential expression of EBV miRNA in different EBV-associated tumors so as to modulate the progression of tumor.

In the process of EBV miRNA biogenesis, Adenosine-to-inosine (A-to-I) editing is involved in modulating the expression and function of EBV miRNAs. A-to-I editing of pri-miR-BART6 leads to down-regulation of mature miR-BART6 expression (Iizasa et al., 2010). Editing of pri-miR-BART3 not only inhibits the biogenesis of mature miR-BART3 but also changes the nucleotide within the seed sequence of mature miR-BART3 and consequently impairs its ability to target DICE1 (Lei et al., 2013a). The editing rate differs in different cell lines. For instance, the nasopharyngeal carcinoma cell line C666-1 has a lower editing rate of pri-miR-BART6 than the EBV-transformed lymphoblastoid cell line GM607 and the Burkitt lymphoma cell line Daudi; these data are consistent with the higher levels of mature miR-BART6 in C666-1 than in GM607 and Daudi cells (Iizasa et al., 2010).

3. Targets and functions of EBV miRNAs

EBV miRNAs have been reported to target both viral and host cell transcripts, contributing to viral replication and latency in the host as well as tumor development and progression. As shown in Fig. 2 and Table 1, a number of EBV miRNA targets have been identified, revealing the multiple functions of EBV miRNAs in EBV-associated tumors.

3.1. Promoting viral replication

SUMOylation, a post-translational modification by the Small Ubiquitin-like Modifier (SUMO), is one of the host defense mechanisms activated when cells are exposed to stresses such as hypoxia, heat shock and viral infection (Manza et al., 2004; Golebiowski et al., 2009; Everett et al., 2013). During viral replication, the products of the immediate early genes in the BamH1 fragment Z left reading frame 1 (*BZLF1*) and BamH1 fragment R left reading frame 1 (*BRLF1*) of EBV are SUMOylated and viral production is inhibited (Hagemeier et al., 2010; Chang et al., 2004). This process is regulated by RNF4, a SUMO-targeted ubiquitin ligase which mediates the ubiquitination and

Table 1
Targets and functions of EBV miRNAs in EBV-associated tumors.

EBV miRNAs	Targets	Effects of miRNAs	References
BHRF1-1	P53	Anti-apoptosis	(Li et al., 2012)
	RNF4	promote viral production	(Li et al., 2017)
BHRF1-2	PRDM1/Blimp1	Anti-apoptosis	(Ma et al., 2016)
	BHRF1	Maintain latency	(Bernhardt et al., 2016)
	IL-12	Immune evasion	(Tagawa et al., 2016)
	Cathepsin B	Immune evasion	(Tagawa et al., 2016)
	AEP	Immune evasion	(Tagawa et al., 2016)
	GILT	Immune evasion	(Tagawa et al., 2016)
BHRF1-3	CXCL-11	Immune evasion	(Xia et al., 2008)
	PTEN	Anti-apoptosis	(Bernhardt et al., 2016)
	TAP	Immune evasion	(Albanese et al., 2016)
BART1-5p	LMP1	Promote cancer development	(Lo et al., 2007)
	PTEN	Promote tumor metastasis	(Cai et al., 2015a)
	IL-12	Immune evasion	(Tagawa et al., 2016)
	Cathepsin B	Immune evasion	(Tagawa et al., 2016)
	AEP	Immune evasion	(Tagawa et al., 2016)
	GILT	Immune evasion	(Tagawa et al., 2016)
BART1-3p	CASP3	Anti-apoptosis	(Vereide et al., 2013; Harold et al., 2016)
BART2-5p	BALF5	Maintain latency	(Barth et al., 2008)
	MICB	Immune evasion	(Nachmani et al., 2009)
	IL-12	Immune evasion	(Tagawa et al., 2016)
	Cathepsin B	Immune evasion	(Tagawa et al., 2016)
	AEP	Immune evasion	(Tagawa et al., 2016)
	GILT	Immune evasion	(Tagawa et al., 2016)
BART3-3p	IPO7	Immune evasion	(Dolken et al., 2010)
BART3-5p	DICE1	Promote cell growth, anti-apoptosis	(Kang et al., 2015; Lei et al., 2013b)
	CASZ1	Anti-apoptosis	(Kang et al., 2015)
BART4-5p	Bid	Anti-apoptosis	(Shinozaki-Ushiku et al., 2015)
BART5-5p	LMP1	Immune evasion	(Verhoeven et al., 2016)
	PUMA	Anti-apoptosis	(Choy et al., 2008)
BART6-5p	Dicer	Maintain latency	(Iizasa et al., 2010)
	OCT1	Anti-apoptosis	(Kang et al., 2015)
BART6-3p	IL-6R	Immune evasion	(Zhang et al., 2017)
	PTEN	Promote proliferation	(Ambrosio et al., 2014; Zhou et al., 2016)
	lncRNA LOC553103	inhibit migration and invasion	(He et al., 2016)
BART7-3p	PTEN	Promote tumor metastasis	(Cai et al., 2014)
	GFPT1	Increase radiation sensitivity	(Gao et al., 2017)
BART8	STAT1	Anti-apoptosis	(Huang and Lin, 2014)
BART9	E-Cadherin	Promote tumor metastasis	(Tsai et al., 2017; Hsu et al., 2014)
BART10-3p	BTRC	Promote tumor metastasis	(Yan et al., 2015)
BART11	FOXP1	Promote proliferation	(Song et al., 2016)
BART13-3p	CAPRIN2	Anti-apoptosis	(Riley et al., 2012)
BART15	BRUCE	promote apoptosis	(Choi et al., 2013)
	TAX1BP1	promote apoptosis	(Choi and Lee, 2017)
	NLRP3	Immune evasion	(Haneklaus et al., 2012)
BART16	CREB-binding protein	Immune evasion, anti-apoptosis	(Hooykaas et al., 2017; Kang et al., 2015)
	TOMM22	Anti-apoptosis	(Dolken et al., 2010)
	CASP3	Anti-apoptosis	(Vereide et al., 2013)
	LMP1	Promote cancer development	(Lo et al., 2007)
	SH2B3	Anti-apoptosis	(Kang et al., 2015)
BART17-5p	LMP1	Promote cancer development	(Lo et al., 2007)
	TAP	Immune evasion	(Albanese et al., 2016)
BART18-5p	MAP3K2	Maintain latency	(Qiu and Thorley-Lawson, 2014)
BART20-5p	T-bet	Anti-apoptosis	(Lin et al., 2013)
	IFN-γ	Anti-apoptosis	(Huang and Lin, 2014)
	BZLF1, BRLF1	Maintain latency	(Jung et al., 2014)
	BAD	Anti-apoptosis	(Kim et al., 2015)
BART22	LMP2A	Immune evasion	(Lung et al., 2009)
	MAP3K5	Anti-apoptosis	(Chen et al., 2017)
	NDRG1	Inhibit differentiation and metastasis	(Kanda et al., 2014)
	CASP3	Anti-apoptosis	(Harold et al., 2016)
	PAK2	Anti-apoptosis	(Kang et al., 2015)
	TP53INP1	Anti-apoptosis	(Kang et al., 2015)

degradation of SUMOylated proteins (Praefcke et al., 2012). A recent study discovered that ebv-miR-BHRF1-1 could enhance viral replication by targeting RNF4 in the late phase of EBV productive infection, which may be a strategy adopted by the virus to counteract the host defense (Li et al., 2017).

3.2. Latency maintaining

BART2-5p was the first EBV miRNA identified to be involved in maintaining viral latency. By reducing the protein expression of viral DNA polymerase BALF5, BART2-5p suppresses the transition from latent to lytic replication (Barth et al., 2008). In EBV, viral replication is triggered by the expression of two immediate early genes *BZLF1* and *BRLF1*. BART20-5p can target both *BZLF1* and *BRLF1* so as to inhibit

lytic induction (Jung et al., 2014). BART18-5p has also been reported to stabilize latency by silencing MAP kinase kinase kinase 2 (MAP3K2), a key mediator of viral replication (Qiu and Thorley-Lawson, 2014). In addition, BART6-5p can specifically target the type III RNase, Dicer, that consequently reduces production of many mature miRNAs and lowers the levels of viral proteins expressed in the lytic cycle, including Zta (encoded by *BZLF1*) and Rta (encoded by *BRLF1*), all of which are favorable for maintaining a latent infection (Iizasa et al., 2010).

3.3. Immune evasion

In healthy individuals, EBV infection is generally asymptomatic and is controlled by host immune system. However, EBV can be latent in host for life because it can form a delicate balance with the host immune system (Wang et al., 2018). In order to establish persistent infection, EBV seems to have evolved sophisticated mechanism to evade the host immune surveillance. EBV miRNAs contribute to the immune evasion by decreasing the expression of its own immunogenic latent proteins and impairing the immune function of the host.

LMP2A is a viral antigen with strong immunogenicity which can be recognized by cytotoxic T cells (CTLs). BART22 has been demonstrated to target LMP2A and facilitate NPC carcinogenesis (Lung et al., 2009). Similarly, another immunogenic viral protein, LMP1, has also been reported to be suppressed by BART Cluster 1 miRNAs, including BART1-5p, 16, 17-5p and 5-5p (Verhoeven et al., 2016; Lo et al., 2007). By inhibiting immunogenic viral protein levels, EBV miRNAs protect the EBV-infected cells from host immune killing and therefore contribute to the development and progression of EBV-associated tumors.

A number of host immune molecules have been identified as targets of EBV miRNAs. C-X-C motif chemokine 11 (CXCL-11) is an IFN-inducible T-cell attracting chemokine. Its expression is significantly repressed by BHRF1-3 miRNA in type III latency cell lines and EBV-positive AIDS-related DLBCLs, and this may weaken the function of CTL cytokine networks (Xia et al., 2008). MHC class I polypeptide-related sequence B (MICB), a stress-induced NK cell ligand, can be down-regulated by BART2-5p and thus the killing by NK cells is reduced (Nachmani et al., 2009). Nuclear importer receptor importin 7 (IPO7) was also confirmed as a target of BART3-3p, resulting in decreased IL-6 secretion and impaired innate immunity (Dolken et al., 2010; Yang et al., 2009). Interleukin-6 receptor (IL-6R) is a receptor of several cytokines such as IFN-α, IL-12 and IL-27, mediating the host response to pathogen and cell proliferation and survival (Kishimoto, 2005; Peters et al., 1996). Recently, BART6-3p and cellular miR-197 were reported to synergistically compromise the host immune defenses by inhibiting their common target IL-6R in BL (Zhang et al., 2017). NOD-like receptor family pyrin domain containing 3 (NLRP3) is another intracellular sensor involved in monitoring extraneous pathogen that can be down-regulated synergistically by EBV miRNA BART15 and cellular miR-223 (Haneklaus et al., 2012). In addition, as a key element of the interferon (IFN) signaling pathway, CREB-binding protein has also been reported to be reduced by BART16, thus hampering the production and function of IFN and consequently interfering with the host immune response to EBV infection (Hooykaas et al., 2017). Moreover, IL-12 (a critical cytokine for differentiation of Th1 cells), AEP and CTSB (both are lysosomal endopeptidases), and GILT (thiol reductase) have been identified as targets of BART1, BART2, and BHRF1-2, resulting in impaired function of cytotoxic EBV-specific CD4 + effector T cells (Tagawa et al., 2016). Meanwhile, a number of EBV miRNAs directly target TAP and IL-12, repressing the activity of EBV-specific CD8 + T cells (Albanese et al., 2016).

Therefore, EBV miRNAs adopts multiple targets and pathways to evade immune surveillance and facilitate establishment of a lifelong infection. However, in immunosuppressed individuals, the balance between EBV and host immune system is broken, so the hosts are prone to develop EBV-associated diseases, especially EBV-associated tumors (Hatton et al., 2014).

3.4. Regulation of apoptosis

As mentioned previously in this review, BHRF1 miRNAs are derived from the *BHRF1* cluster, a region encoding *BHRF1* transcript, which is also translated into BHRF1 protein, a homolog of the anti-apoptotic protein Bcl-2. In the early stage of EBV infection, BHRF1 protein is highly expressed and the expression of phosphatase and tensin homolog deleted on chromosome ten (PTEN) is directly inhibited by miR-BHRF1-3. This leads to a reduction in apoptosis and may facilitate the viral replication. However, in the late stage, BHRF1 protein expression is dramatically reduced by miR-BHRF1-2, which favors long term latent infection of the host (Bernhardt et al., 2016). miR-BHRF1-2 also suppresses apoptosis by targeting tumor suppressor PRDM1/Blimp1, thus facilitating the growth of EBV-infected B cells and contributing to the development of B-cell lymphoma (Ma et al., 2016). In addition, miR-BHRF1-1 can target P53 to inhibit apoptosis. In NPC, the absence of miR-BHRF1-1 promotes the maintenance of EBV latent infection (Li et al., 2012).

Of the BART miRNAs, BART5 was the first one identified to prevent apoptosis by targeting P53 up-regulated modulator of apoptosis (PUMA), a pro-apoptotic BH3-only member of the Bcl-2 family, thus inhibiting the p53-independent apoptosis in NPC and EBVaGC (Choy et al., 2008). Subsequently, BART16 was demonstrated to suppress Bcl-2 associated X (BAX)-induced apoptosis by targeting translocase of outer mitochondrial membrane 22 (TOMM22), a transporter responsible for importing BAX from cytosol to mitochondria (Dolken et al., 2010). In addition, BART miRNAs of cluster 1 were found to reduce levels of the pro-apoptotic protein Bcl-2 interacting mediator of cell death (Bim) (Marquitz et al., 2011). BART13-3p, BART20-5p and BART4-5p were also shown to decrease expression of the pro-apoptotic protein caprin family member 2 (CAPRIN2) (Riley et al., 2012), T-bet (Lin et al., 2013), and BH3-interacting domain death agonist (Bid) (Marquitz et al., 2014), respectively. BART16 also targets caspase 3 (CASP3), a central executioner in the process of apoptosis (Vereide et al., 2013), that can also be directly reduced by BART22, BART1-3p and BART2-5p (Harold et al., 2016). BART20-5p also inhibits apoptosis by suppressing multiple pro-apoptotic proteins, including Bcl-2-associated death promoter (BAD) (Kim et al., 2015), IFN-γ and signal transducers and activators of transcription (STAT1) (Huang and Lin, 2014). IFN-γ and STAT1 induce apoptosis directly or indirectly through activating P53 (Vilcek, 2003; Bhinge et al., 2007), and they are also targets for BART8 (Huang and Lin, 2014). Recentely, BART22 was also reported to exert an anti-apoptosis function by targeting MAP kinase kinase kinase 5 (MAP3K5), a tumor suppressor that facilitates apoptosis (Chen et al., 2017). Moreover, using photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP), Kang et al. (2015) identified seven anti-apoptotic mRNAs as targets of BART miRNAs, including CASZ1 and DICE1 (BART3), OCT1 (BART6), SH2B3 and CREBBP (BART16), and PAK2 and TP53INP1 (BART22).

Not all EBV miRNAs inhibit apoptosis. BART15-3p was surprisingly found to promote apoptosis by silencing baculovirus inhibitor of apoptosis repeat-containing ubiquitin-conjugating enzyme (BRUCE) (Choi et al., 2013) and Tax1-binding protein 1 (TAX1BP1) (Choi and Lee, 2017). The significance of BART15-3p inducing apoptosis is still unknown. It may be conducive to accelerate the viral lytic cycle and facilitate the spreading of virus between the host cells (Richard and Tulasne, 2012). Nevertheless, considering the low level of BART15-3p in lymphoma and intermediate level in epithelial malignancy, and the fact that many other EBV miRNAs function as apoptosis inhibitors, the pro-apoptotic effect of BART15-3p would be mild in EBV-associated tumors, and the overall impact of EBV miRNAs on the apoptosis of host cells would be negative (Choi et al., 2013).

3.5. Regulation of cancer metabolism

Deregulating cellular energetics is one of hallmarks of cancer

(Hanahan and Weinberg, 2011). Through gene expression profiling analysis and qRT-PCR confirming in NPC samples, Ye et al. (2013) demonstrated that BART1 significantly up- and down-regulated a number of metabolism-associated genes, including G6PD, SAT1, ASS1, PAST1, FUT1, SGPL1, DHRS3, B4GALT1, PHGDH, IDH2, PISD, UGT8, LDHB and GALNT1, indicating that BART1 has a role in the regulation of cancer metabolism, although its direct target genes and its impact on metabolic processes still need to be elucidated.

3.6. Promoting tumor progression

Accumulating evidence has indicated that EBV miRNAs play important roles in the progression of EBV-associated tumors by targeting tumor-suppressing genes of the host cell.

Several EBV miRNAs confer a growth advantage to EBV-associated tumors through repressing the anti-proliferation genes. BART3* has been shown to promote NPC cell growth by targeting the tumor suppressor determination of interleukin 4 commitment 1 (DICE1) (Lei et al., 2013b). BART11, including BART11-3p and BART11-5p, induces cancer cell proliferation in NPC and GC by specifically suppressing forkhead box P1 (FOXP1) expression (Song et al., 2016). In addition, BART6-3p promotes cell proliferation and inhibits cell death by down-regulating the expression of IL-6R and PTEN in BL (Ambrosio et al., 2014), and this effect can be synergistically enhanced by cellular miRNA miR-142 (Zhou et al., 2016). Besides, BART9 has also been shown to enhance growth of nasal NK/T-cell lymphoma cell by up-regulating LMP1 levels through an unknown mechanism (Ramakrishnan et al., 2011).

Other EBV miRNAs promote tumor progression by facilitating metastasis. BART9 has been confirmed to induce epithelial-mesenchymal transition (EMT) by directly targeting E-cadherin in NPC and EBVaGC so as to promote metastasis (Tsai et al., 2017; Hsu et al., 2014). BART7-3p and BART1-5p have also been reported to specifically bind to the 3′UTR of PTEN, an important human tumor suppressor, and thus enhance the metastasis of NPC (Cai et al., 2014, 2015a). In addition, BART10-3p induces invasion and metastasis in NPC by inhibiting BTRC, an inhibitor of the Wnt signaling pathway, which is involved in the ubiquitination and degradation of β-catenin (Yan et al., 2015). A number of other negative regulators of the Wnt pathway, including Wnt inhibitory factor 1 (WIF1), Nemo-Like Kinase (NLK) and adenomatous polyposis coli (APC), have been predicted to be targets of EBV BART miRNAs. Western blot analysis showed that WIF1 was downregulated by BART19-3p, that APC expression was reduced by BART19-3p, BART7 and BART17-5p, and that the expression of NLK was decreased by BART19-3p, BART14 and BART18-5p (Wong et al., 2012). But whether WIF1, APC and NLK are the direct targets of these EBV miRNAs remains to be determined. In addition, BART22 inhibits the differentiation and promotes the metastasis of NPC cells by targeting N-myc downstream regulated gene 1 (NDRG1), another tumor-suppressor mainly expressed in epithelial cells (Kanda et al., 2014).

Intriguingly, EBV miRNAs are not always oncogenic. BART6-3p has been reported to target an invasion and metastasis promoting lncRNA LOC553103 in NPC and GC (He et al., 2016), suggesting the interaction between EBV miRNAs and the host might be very complex.

3.7. Regulation of sensitivity to chemotherapy and radiation

As a pro-apoptotic miRNA, BART15-3p not only can induces apoptosis in GC through targeting BRUCE and TAX1BP1 but can also increase chemosensitivity of GC cells to 5-Fluorouracil (Choi et al., 2013; Choi and Lee, 2017). BART7 has also been reported to be able to enhance the radiation sensitivity by targeting glutamine-fructose-6-phosphate transaminase 1 (GFPT1) (Gao et al., 2017), a key rate-limiting enzyme of the process of controlling transforming growth factor β1 (TGFβ1) (Schleicher and Weigert, 2000). TGFβ1 is a crucial cytokine for induction of self-renewal and repair of damaged cells (Kolm-Litty et al., 1998). Suppression of GFPT1 by BART7 leads to reduction of TGFβ1 and eventually increases the sensitivity of NPC cells to radiotherapy.

4. EBV miRNAs as biomarkers

In the histologically clear resection margin of recurrent NPC, positive BART7 expression is significantly associated with higher risk of local tumor relapse. This subgroup of patients may need postoperative adjuvant therapy to prevent the recurrence (Chan et al., 2015). In EBVaGC, higher expression of BART20-5p in tumor tissues is an independent predictor of poor recurrence-free survival (Kang et al., 2017). In addition, an analysis of the Cancer Genome Atlas (TCGA) miRNA data across many tumor types including NPC and GC showed that an EBV miRNA cluster that includes BART2, BART4, BART5, BART18 and BART22, is associated with a more aggressive phenotype and poor outcome (Pandya et al., 2015). These findings indicate that the expression level of EBV miRNAs in tumor samples may serve as a useful predictive biomarker for the prognosis of EBV-associated tumors.

Increasing evidence has shown that EBV miRNAs can be released from EBV infected cells into blood, facilitating the transfer of EBV miRNAs from malignant to non-malignant cells (Pegtel et al., 2010; Gourzones et al., 2010). In NPC, BART3, BART7 and BART13 have been detected in plasma at abundant levels but rarely found in the non-NPC group or healthy controls, suggesting these EBV miRNAs may be useful diagnostic biomarkers for NPC. Furthermore, a reduction of BART7 and BART13 levels in plasma after radiotherapy makes them potential predictors of treatment efficacy (Zhang et al., 2015). Another study demonstrated that BART2-5p, BART17-5p and BART18-5p were present in the serum of NPC patients. BART17-5p levels decreased significantly in patients in remission after treatment but were still detected at higher levels in patients with relapse or residual tumors, indicating BART17-5p is a candidate biomarker for treatment outcomes (Hirai et al., 2016). Similarly, higher levels of BART2-5p, BART7-3p, BART13-3p and BART1-5p also have been detected in the serum of patients with nasal NK/T-cell lymphoma compared with healthy controls, and the high BART2-5p level is significantly correlated with disease progression and poor survival rate (Komabayashi et al., 2016).

5. EBV miRNAs as therapeutic targets

Given the multiple and crucial roles of EBV miRNAs in tumor initiation and progression, they are believed to be promising targets for cancer treatment. A few attempts have been made to target EBV miRNAs as a therapy for EBV-associated malignancies. BART7-3p is abundantly expressed in NPC, and its pro-tumor effect has been confirmed in a number of studies (Cai et al., 2014; Gao et al., 2017; Chan et al., 2015; Zhang et al., 2015). Hence, Cai et al. (2015b) developed a gold-nanoparticle carrying anti-ebv-miR-BART7-3p which can significantly inhibit the growth of NPC in a mouse model, providing a potentially effective treatment for NPC. Compared with targeting individual EBV miRNA, knocking down a cluster of EBV miRNAs may be more effective. Yuen et al. (2015) has used a CRISPR/Cas9 system to remove the P1 and P2 promoters driving *BART* gene expression and has successfully abrogated the expression and activity of BART miRNAs in NPC and EBVaGC cell lines.

Admittedly, miRNA-based therapeutics is still in the initial stages and its effectiveness and safety warrants more validation *in vitro* and *in vivo*. However, the technology is developing rapidly. The anti-miR-122 oligonucleotide Miravirsen is the first miRNA-targeting drug for treatment of hepatitis C infection, and it has passed the phase II clinical trial successfully (Janssen et al., 2013) and entered the phase III clinical trial. Another miRNA drug MRX34, a synthetic miR-34 mimic loaded into liposomal nanoparticles for the treatment of liver cancer (Bader, 2012), recently entered phase II clinical trial (Schmidt, 2017). Therefore, despite the fact that the development of miRNA-targeting drug is

still a challenge due to their poor delivery, inefficient cell permeability and off-target effect (Schmidt, 2017), with the technological progress being made, EBV miRNA-targeting therapeutics is promising and is believed to confer great benefit to the treatment of EBV-associated tumors.

6. Conclusion

Although EBV is thought to be associated with a number of human cancers, the pathogenic mechanism of EBV remains unclear. Some viral proteins such as LMP1, LMP2A and EBNA1, have been shown to have oncogenic properties (Dawson et al., 2012; Frappier, 2012). Interestingly, several studies in more recent years have shown that non-coding miRNAs from EBV are also responsible for the development and progression of EBV-associated tumors. The expression levels of EBV miRNAs are finely tuned at different infection stages and in different types of tumors. Through targeting both viral and host genes, EBV miRNAs exert their functions by modulating various biological processes, including viral replication, latency maintenance, immune escape, cell proliferation, apoptosis, metabolism, and tumor metastasis. In addition, several EBV miRNAs have been demonstrated to serve as biomarkers for diagnosis, prognosis and treatment efficacy of malignancies. With the identification of new target genes, more roles of EBV miRNAs will be found, and the development of EBV miRNA-targeting therapeutics will certainly lead to a profound revolution for the treatment of EBV-associated tumors in the future.

Conflicts of interest

All the authors declare that they have no conflict of interest.

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