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REVIEW
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Role of Virally Encoded Circular RNAs in the Pathogenicity of Human Oncogenic Viruses

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Human oncogenic viruses are a group of important pathogens that etiologically contribute to at least 12% of total cancer cases in the world. As an emerging class of non-linear regulatory RNA molecules, circular RNAs (circRNAs) have gained increasing attention as a crucial player in the regulation of signaling pathways involved in viral infection and oncogenesis. With the assistance of current circRNA enrichment and detection technologies, numerous novel virally-encoded circRNAs (vcircRNAs) have been identified in the human oncogenic viruses, initiating an exciting new era of vcircRNA research. In this review, we discuss the current understanding of the roles of vcircRNAs in the respective viral infection cycles and in virus-associated pathogenesis.

Keywords: circular RNA, circRNA, oncogenic virus, EBV, KSHV, HPV, MCV, HBV

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INTRODUCTION

Circular RNAs (circRNAs) are a class of non-linear macromolecules that are single-stranded covalently closed cyclized RNAs with a great diversity in length, varied from less than 100 to thousands of nucleotides (Kolakofsky, 1976; Sanger et al., 1976; Hsu and Coca-Prados, 1979; Arnberg et al., 1980; Salzman et al., 2012, 2013; Jeck et al., 2013; Memczak et al., 2013; Jeck and Sharpless, 2014; Rybak-Wolf et al., 2015; Kristensen et al., 2018). The first evidence of circRNA was reported in 1976 when Sanger et al. conducted the electron microscopy analysis of the denatured RNA molecules from the viroid of the pale fruit disease of cucumber (CPFV). The authors found that these viroid RNA existed as single-stranded circles (Sanger et al., 1976). Three years later, endogenous circRNAs were further detected in the cytoplasmic RNA extracted from human HeLa cells, monkey CV-1 cells, and Chinese hamster ovary cells as well as total RNA isolated from *Physarum polycephalum* (a plasmodial slime mold) (Hsu and Coca-Prados, 1979). However, at that time, the synthesis of circRNAs was considered to be a rare event and those circRNAs were treated as "splicing noise" and byproducts likely resulting from splicing errors (Cocquerelle et al., 1993). Hence, the potential impact of this discovery did not receive broad recognition and only a handful of follow-up studies were conducted in the next three decades.

The dawn of the circRNA era occurred approximately 30 years later when high-throughput next-generation sequencing technology and associated informatics approaches have been utilized in the circRNA research field. Since the early 2010s, thousands of endogenous circRNAs have been identified in all domains of life including human, animals, plants, yeasts,

bacteria, and viruses (Danan et al., 2012; Salzman et al., 2012, 2013; Jeck et al., 2013; Memczak et al., 2013; Jeck and Sharpless, 2014; Rybak-Wolf et al., 2015; Kristensen et al., 2018; Tagawa et al., 2018; Toptan et al., 2018; Ungerleider et al., 2018, 2019a; Zhao et al., 2019). To date, the list of identified circRNAs is still rapidly growing and circRNAs are now appreciated as an important evolutionarily conserved component of living organisms.

Circular RNAs are usually expressed in a tissue- and/or developmental stage-specific manner (Memczak et al., 2013). The mature circRNAs can be detected in cytoplasm, nucleus, as well as extracellular vesicles (e.g., exosomes) (Li Y. et al., 2015). In general, circRNAs show ∼100–1,000 times lower abundance than their associated isogenic linear RNAs and are generated by more than 20% of expressed genes (Huang et al., 2017). However, in many cases, circRNAs can be the main gene products and exhibit more than 10-fold higher expression levels compared to related linear RNAs (Jeck et al., 2013). Lacking free termini, circRNAs are resistant to the hydrolysis by numerous cellular exonucleases such as RNase R, and thus have significantly longer half-lives compared to their linear RNA counterparts (Jeck et al., 2013).

Based on the parental gene component that's carried by the circRNAs, circRNAs can be classified to four major types (**Figure 1**)(Jeck et al., 2013; Zhang et al., 2013; Li Z. et al., 2015; Noto et al., 2017): (1) the most common type is the exonic circRNAs (ecircRNAs) which contain only one or multiple exons; (2) the exon-intron circRNAs (EIciRNAs) carry both exons and introns; (3) the circular intronic RNAs (ciRNAs) carry introns only; and (4) the tRNA intronic circRNAs (tricRNAs) are formed by circularization of the excised tRNA introns. The detailed mechanisms of generating different types of circRNAs are still less understood. The current theory holds that the formation of ecircRNAs and EIciRNAs requires a special type of splicing known as backsplicing, which allows a downstream 5′ splice donor to react with an upstream 3′ splice acceptor. Thus, a 3′–5′ phosphodiester bond can be established to circularize the RNA molecule (Jeck et al., 2013; Li Z. et al., 2015). Unlike exon-derived circRNAs, ciRNAs are generated by lariat introns removed from pre-mRNAs by canonical splicing. A consensus 7-nucleotide GU-rich sequence near the 5′ splice donor and an 11-nucleotide C-rich sequence near the branchpoint site can help prevent the lariat from debranching and exonuclease attacking. Stable ciRNAs can then be formed after trimming the 3′ tail downstream from the branchpoint (Zhang et al., 2013). Lastly, tricRNAs are formed by directly linking the free ends of introns excised from the pre-tRNAs (Noto et al., 2017).

In general, the exon-derived circRNAs (ecircRNAs and EIciRNAs) are produced from pre-mRNA transcribed by RNA polymerase II. The circularization signals are located within the introns adjacent to the circularizable exons (Jeck et al., 2013; Zhang et al., 2014). A growing number of RNA-binding proteins (RBPs) as well as spliceosome components are reported to be involved in the circularization process, including adenosine deaminases acting on RNA (ADAR), the splicing factor muscleblind (MBL), quaking (QKI), DExH-Box helicase 9 (DHX9), epithelial splicing regulatory protein 1 (ESRP1), FUS, serine/arginine-rich proteins, nuclear factors NF90/NF110, small ribonucleoprotein particle U1 subunit 70K (snRNP-U1-70K), small ribonucleoprotein particle U1 subunit C (snRNP-U1-C), pre-mRNA processing 8 (Prp8), cell division cycle 40 (CDC40) (Chen and Yang, 2015; Conn et al., 2015; Ivanov et al., 2015; Kramer et al., 2015; Rybak-Wolf et al., 2015; Aktas et al., 2017; Errichelli et al., 2017; Yu et al., 2017).

In recent years, the way in which circRNAs execute their function has been the subject of active study. Although the biological significance of the majority of the identified circRNAs are still unclear, it's believed that circRNAs predominantly function as regulatory non-coding RNAs (ncRNAs) (Guo et al., 2014). circRNAs can function *in trans* or *in cis*. For instance, there is increasing evidence showing that some circRNAs (such as CDR1as/ciRS-7) can serve as microRNA (miRNA) sponges to sequester endogenous miRNAs and modulate miRNA function *in vivo* (Hansen et al., 2013; Piwecka et al., 2017; Kleaveland et al., 2018). Meanwhile, Ashwal-Fluss et al. (2014) have shown that some circRNAs can be co-transcribed with their associated linear mRNAs in a competitive manner. Thus, these circRNAs can function as an RNA trap for mRNA synthesis by competing with linear splicing *in cis*. Since circRNAs can bind to numerous RBPs, it's postulated that these stable RNA molecules can serve as decoys, scaffolds, or transporters for many RBPs and regulate their functions (Du et al., 2016; Holdt et al., 2016; Abdelmohsen et al., 2017; Pamudurti et al., 2018). The discovery of circRNAs in the extracellular compartments such as exosomes indicates that circRNAs might also function as secretory intercellular signaling molecules to affect distant cells (Li Y. et al., 2015). Interestingly, several recent studies have shown that certain circRNAs (e.g., circZNF609) can be translated in a non-canonical cap-independent manner (Legnini et al., 2017; Pamudurti et al., 2017; Yang et al., 2017). Although the biological impact of circRNA translation and the resulting products are still largely unclear, it's postulated that the truncated protein products/peptides may compete with the full-length protein products encoded by their associated linear mRNAs and affect relevant signaling pathways (Legnini et al., 2017). Together, this evidence suggests that circRNAs may function as important gene regulators and modulate signaling transduction at different levels.

Cancer is developed through a multi-step and multi-factorial process. As an emerging gene regulator, circRNAs are expected to be involved in the regulation of various carcinogenic pathways. To date, there is emerging evidence showing that disruption of circRNA expression correlates with a broad spectrum of human cancers, including hepatocellular carcinoma (HCC; Qin et al., 2016; Shang et al., 2016; Yu et al., 2016; Xu et al., 2017; Yao et al., 2017), colorectal cancer (Huang et al., 2015; Wang et al., 2015; Guo et al., 2016; Xie et al., 2016; Hsiao et al., 2017; Weng et al., 2017; Zhu et al., 2017), gastric cancer (Li P. et al., 2015, 2017; Chen J. et al., 2017; Chen S. et al., 2017; Li W.H. et al., 2017; Sui et al., 2017; Zhao et al., 2018), gliomas (Barbagallo et al., 2016; Zheng et al., 2017), lung cancer (Wan et al., 2016; Zhang et al., 2020), breast cancer (Liu Z. et al., 2019), pancreatic ductal adenocarcinoma (Li et al., 2016), laryngeal

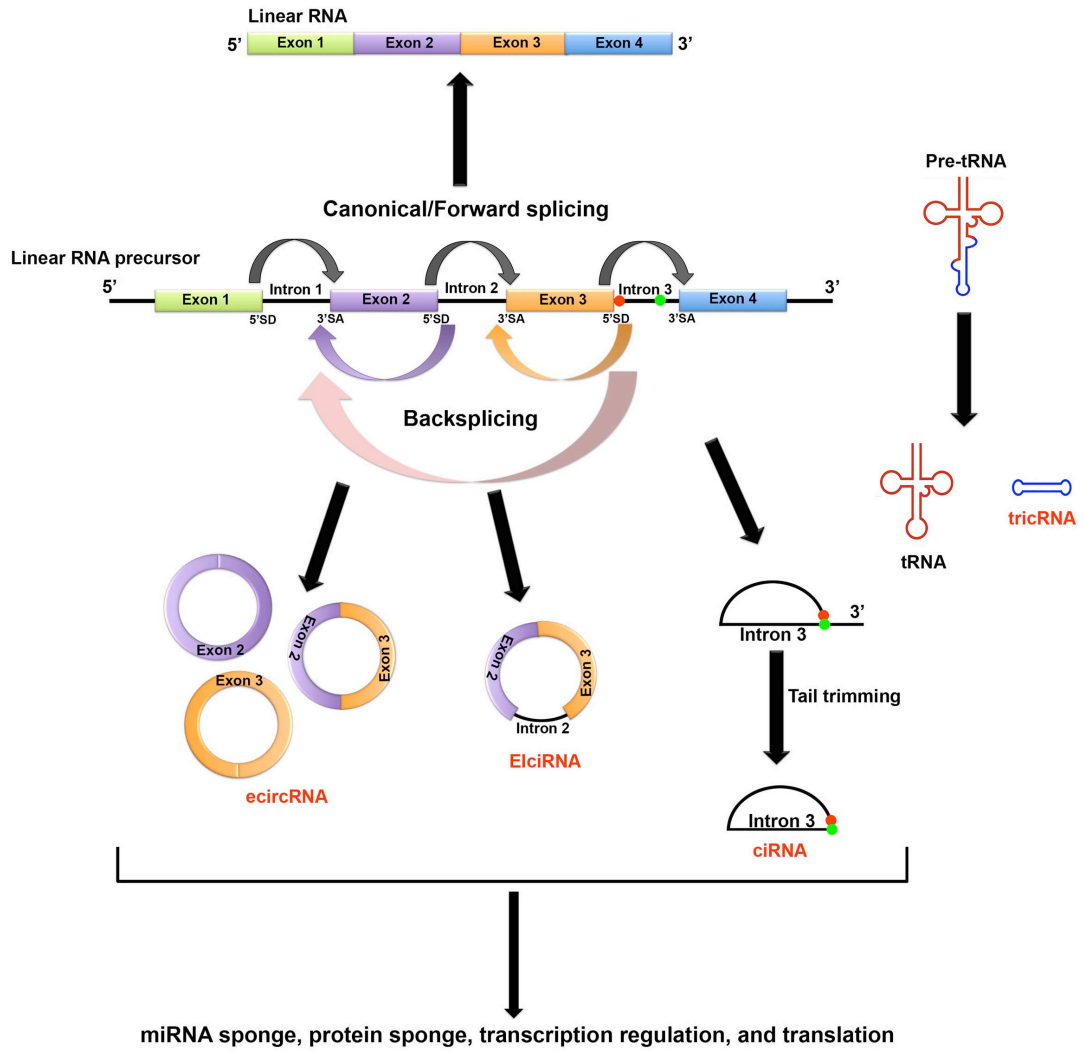

FIGURE 1 | Biogenesis and potential functions of circRNAs. Based on the parental gene component that's carried by the circRNAs, circRNAs can be classified to four major types. (1) The most common type is the exonic circRNAs (ecircRNAs) which contain only one or multiple exons; (2) The exon-intron circRNAs (ElciRNAs) carry both exons and introns; (3) The circular intronic RNAs (ciRNAs) carry introns only; and (4) The tRNA intronic circRNAs (tricRNAs) are formed by circularization of the excised tRNA introns. The formation of ecircRNAs and ElciRNAs requires a special type of splicing known as backsplicing, which allows a downstream 5′ splice donor (SD) to react with an upstream 3′ splice acceptor (SA). Thus, a 3′–5′ phosphodiester bond can be established to circularize the RNA molecule. ciRNAs are generated by lariat introns removed from pre-mRNAs by canonical splicing. A consensus 7-nucleotide GU-rich sequence near the 5′ splice donor (shown as red dot in the figure) and an 11-nucleotide C-rich sequence near the branchpoint site (shown as green dot in the figure) can help prevent the lariat from debranching and exonuclease attacking. Stable ciRNAs can then be formed after trimming the 3′ tail downstream from the branchpoint. Lastly, tricRNAs are formed by directly linking the free ends of introns excised from the pre-tRNAs. circRNAs can function as molecular sponges to sequester miRNA and protein. In general, circRNAs mainly function as non-coding regulatory transcripts. However, some circRNAs can be translated through either IRES-mediated or m^6A-mediated non-canonical cap-independent translation initiation.

cancer (Xuan et al., 2016), esophageal squamous cell carcinoma (Xia et al., 2016), basal cell carcinoma (Sand et al., 2016b), cutaneous squamous cell carcinoma (Sand et al., 2016a), ovarian carcinoma (Ahmed et al., 2016), cervical cancer (Wu et al., 2020), and nasopharyngeal carcinoma (NPC; Yang et al., 2020). During the oncogenesis process, circRNAs can serve as either oncogenes or tumor suppressors by inhibiting critical protein-coding and/or non-coding genes involved in oncogenesis. Currently, the study of detailed mechanisms through which circRNAs facilitate cancer development is still in its infancy. Nevertheless, it is apparent that the discovery of circRNAs has created a new dimension for us to elucidate the mechanism of carcinogenesis.

ONCOGENIC VIRUSES, vcircRNAs, AND CANCERS

The family of human oncogenic viruses currently comprises seven members: Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), human papillomavirus (HPV),

Merkel cell polyomavirus (MCV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-lymphotropic virus (also known as human T-cell leukemia virus type 1). Together, they account for 12–16% of total cancer cases in the world (White et al., 2014; Zapatka et al., 2020). Notably, only a small portion of infected persons develop cancers, which usually happens many years after initial infection (Mesri et al., 2014). It indicates that these oncogenic viruses are not fully oncogenic – they are necessary but not sufficient to cause cancers (Mesri et al., 2014). Indeed, for all these oncogenic viruses, cancer formation is believed to be a biological accident, but not an adaptation during their co-evolution with the hosts (Moore and Chang, 2017). After all, cancers are considered as evolutionarily dead-end events which put both hosts and viruses in danger (Moore and Chang, 2017). Viral transmission is the central point for the viral persistence and evolution. However, oncogenic viruses carried by these cancers are usually not permissive to produce sufficient infectious virions in order to support human-to-human transmission (Moore and Chang, 2017). The transmission of the oncogenic virus generally occurs among asymptomatic individuals. The dramatic increase of viral cancers in the immunocompromised individuals indicates that host immune surveillance plays a critical role in preventing viral cancer formation in the infected individuals (Moore and Chang, 2017).

So, why do these oncogenic viruses cause cancers? There is a continual battle between hosts and oncogenic viruses. Hosts have evolved various innate and adaptive immune strategies to eliminate viral infections. Meanwhile, oncogenic viruses have co-evolved strong immunoevasion and replication programs to counteract hosts' defense systems (Mesri et al., 2014). During the process of immunoevasion and replication, some of the viral strategies such as proliferation and anti-apoptosis accidentally break host homeostasis and trigger cancer development. However, since the cancer development is a multifactorial and multistep process, additional co-factors such as chronic inflammation, environmental mutagens and immunosuppression are usually required for the formation of viral cancers (Mesri et al., 2014).

To date, increasing evidence has indicated that circRNAs play important roles in the regulation of RNA biogenesis and cell signaling. We are not surprised to see oncogenic viruses once again exploit these pathways to suit their needs of survival. Next, we have discussed a number of recently identified vcircRNAs and the possible relevance of these vcircRNA in virus infection and the development of virus-associated human malignancies.

EPSTEIN-BARR VIRUS

Epstein-Barr virus is also known as EBV or human herpesvirus 4. It is the first human tumor virus identified in 1964 and accounts for ∼200,000 new cancer cases annually (Rickinson and Kieff, 2007; Young et al., 2016; Shannon-Lowe and Rickinson, 2019). EBV is etiologically related to a number of human malignant diseases including Burkitt's lymphoma (BL), Hodgkin's disease (HD), extranodal nasal-type natural killer/T cell lymphoma (NKTL), posttransplant lymphoproliferative disease (PTLD) and lymphoma in AIDS and immunocompromised individuals, NPC, gastric carcinoma (GC), as well as lung cancers (LC) (de Sanjose et al., 2007; Rickinson and Kieff, 2007; Strong et al., 2013a,b; Young et al., 2016; Kheir et al., 2019; Shannon-Lowe and Rickinson, 2019).

Epstein-Barr virus belongs to the human γ-herpesvirus subfamily, in the same family with KSHV. There are two distinct stages in EBV's life cycle: latency and lytic cycle (also known as lytic reactivation). After primary infection, EBV co-exists with its host permanently and exhibits a life-long latency. During the latency, the viral genome remains as an episomal chromosome and only a limited repertoire of viral genes are expressed. In response to certain physiological stimuli, the latent genome can be reactivated to express more than 100 viral genes and produce new infectious virions.

Infectious virions can be transmitted through various types of body fluids, but they are mainly spread through saliva exchange. There's no anti-EBV vaccine currently available and it's estimated that ∼90% of the world population permanently carries EBV (Rickinson and Kieff, 2007). To date, the detailed mechanism of EBV primary infection is still unclear. The current theory holds that EBV initially infects replication-permissive oropharyngeal epithelial cells in the host. The propagated infectious virions are then transmitted from the initial epithelial cells to the infiltrating B-cells in which the full set of viral latency transcripts are expressed (known as type III latency) (**Table 1**). The type III latency can subsequently activate B cells and cause a transient expansion of the EBV-infected B-cell pool. Majority of such B-cells will be eliminated by host immune surveillance. However, a sub-set of infected circulating B-cells can persist for the lifetime of the host. This is attributable to expression of no (type 0 latency) or only one viral protein (type I latency) (**Table 1**). It is believed that sporadic reactivation occurs in epithelial tissues as well as B cells differentiating into plasma cells (Laichalk and Thorley-Lawson, 2005; Sun and Thorley-Lawson, 2007), and the progeny infectious virions are secreted to the saliva and then transmitted to new hosts.

Previously, EBV has been shown to utilize non-coding linear RNAs such as miRNAs, long non-coding RNAs (lncRNAs), and small non-coding EBV-encoded RNAs (EBERs) to facilitate its life cycle and oncogenesis (Pfeffer et al., 2004; Cai et al., 2006; Hutzinger et al., 2009; Concha et al., 2012; Moss and Steitz, 2013; O'Grady et al., 2014; Cao et al., 2015a,b; O'Grady et al., 2016). Recently, increasing evidence has shown that EBV also encodes its own set of circRNAs to promote its survival. Since both the virus survival and tumor survival often share the same needs, the expression of EBV-encoded circRNAs can potentially contribute to the development of virus-associated cancers through the circRNA-dependent alteration of cancer pathways.

Epstein-Barr Virus Encoded circRNAs

Human-to-human transmission is pivot to viral evolution, which requires establishment of long-term infection. Thus, powerful immunoevasion strategies are always needed for the viruses to accomplish their goal of persistence. Since circRNAs are largely non-immunogenic, vcircRNAs are viewed as ideal signal molecules to fulfill many virus needs by regulating host pathways without eliciting the adaptive/innate anti-viral immune responses. This may partially explain why more

TABLE 1 | EBV linear transcripts in different types of latency.

Latency type	Linear viral transcripts	Example of EBV associated malignancies
0	EBER1, EBER2, RPMS1, and viral miRNAs	Memory B cells in EBV(+) individuals
I	EBER1, EBER2, RPMS1, viral miRNAs, and EBNA1	Burkitt's lymphoma
II	EBER1, EBER2, RPMS1, viral miRNAs, EBNA1, LMP1, LMP2A, and LMP2B	Nasopharyngeal carcinoma Hodgkin's disease
III	EBER1, EBER2, RPMS1, viral miRNAs, EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, EBNA-LP, LMP1, LMP2A, and LMP2B	AIDS-associated lymphoma

abundant vcircRNAs are observed in herpesviruses-infected cells. Furthermore, the circRNA biogenesis cascade is likely preferentially suited to DNA viruses. Hence, on one hand, the nuclear replication of these viruses allows them to easily access to many RBPs/splicesome components required for circRNA synthesis. On the other hand, the inherent bicistronic or polycistronic nature of viral transcripts also increases their chance of being backspliced by the splicesomes.

To date, vcircRNAs have been discovered in the transcriptomes of a number of human and non-human viruses including EBV, KSHV, HPV, HBV, MCV, rhesus macaque lymphocryptovirus (rLCV), murid herpesvirus 68 (MHV68), and rat polyomavirus 2 (RatPyV2) (Sekiba et al., 2018; Toptan et al., 2018; Ungerleider et al., 2018, 2019a; Zhao et al., 2019; Abere et al., 2020a,b). As shown in **Table 2**, the herpesvirus family holds the record for the highest number of vcircRNAs. Intriguingly, EBV is the first oncogenic human virus reported to express its own circRNAs and is also one of the top vcircRNA encoders.

Two research teams almost simultaneously utilized the RNase R-sequencing approach to assess vcircRNA expression in a variety of EBV infected cell models and patient tumor samples. In RNase R-sequencing, ribodepleted RNAs are treated with RNase R to remove the linear RNAs before library preparation. Thus, the sensitivity of circRNA detection is improved by enriching the circRNA species. Together, more than 30 EBV-encoded circRNAs (i.e., virally-encoded exonic circRNA and Exon-Intron circRNA) were identified, based on the unique backsplice junctions (Toptan et al., 2018; Ungerleider et al., 2018). Many of these vcircRNAs are expressed at comparable or higher levels to most cellular circRNAs in EBV-associated cancers including gastric cancer, NPC, BL, PTLD, and AIDS-associated lymphoma, indicating a potential functional significance of these vcircRNAs in EBV's pathogenesis (Toptan et al., 2018; Ungerleider et al., 2018; Huang et al., 2019; Liu Q. et al., 2019; Qiao et al., 2019; Gong et al., 2020).

These identified vcircRNAs can be further grouped into 4 clusters. Below, we will discuss each cluster based on their parental gene loci.

RPMS1 Locus

A total of sixteen vcircRNAs were derived from the RPMS1 gene locus within the BamHI A rightward transcript (BART) region (Toptan et al., 2018; Ungerleider et al., 2018). The viral BART gene was initially discovered in EBV positive NPC cells (Hitt et al., 1989). Later on, BART transcripts were observed in both healthy viral carriers and patient with EBV-associated malignancies such as BL, HD, and gastric cancer (Deacon et al., 1993; Chiang et al., 1996; Sugiura et al., 1996; Tao et al., 1998; Webster-Cyriaque and Raab-Traub, 1998). In General, BART region is highly conserved among EBV strains except the B95-8 laboratory EBV strain has a 12-kb deletion of BART locus (Raab-Traub et al., 1980). BART transcripts are a set of highly diverse transcripts including long noncoding RNAs such as RPMS1 and more than 40 EBV viral miRNA spliced from the RPMS1 intronic sequences (Edwards et al., 2008; Yamamoto and Iwatsuki, 2012; Cao et al., 2015b). Both RPMS1 and viral miRNAs are involved in regulating cancer-related signaling pathways.

Four vcircRNAs derived from this locus were observed in all the examined latency types and virally infected cancer biopsies, but not in the B95-8 EBV infected cells due to a BART/RPMS1 locus deletion. Among them, circRPMS1_E4_E2_E3a_I3a_E3b (circBART_1.1) and circRPMS1_E4_E2_E3a_E3b (circBART_1.2) were formed by backsplicing of RPMS1 exon 4 to exon 2. Whereas, circRPMS1_E4_E3a_I3a_E3b (circBART_2.1), circRPMS1_E4_E3a_E3b (circBART_2.2) were formed by backsplicing of RPMS1 exon 4 to exon 3a. Interestingly, the intron-retained vcircRNAs circBART_1.1 and circBART_2.1 show nuclear distribution. In contrast, the exonic circBART_1.2 and circBART_2.2 are localized in both cytoplasm and nucleus.

EBNA Latency Locus

Three vcircRNAs are derived from the EBNA latency locus (Toptan et al., 2018; Ungerleider et al., 2018). Among them, circEBNA_W1_C1 and circEBNA_W2_C1 are formed by backsplicing of Bam HI W repeat W1 and W2 exons to the C1 exon. The expression of EBNA C1 exon is driven by the Bam HI C promoter (Cp) during the type III latency. Thus, these 2 vcircRNAs were mainly detected in type III latency cells with active Cp activity, but not in the Type I or II latency cells with epigenetically-silenced Cp. Meanwhile, circEBNA_U is generated by backsplicing of the Bam HI U fragment exon. Since the expression of EBNA U exon can be driven by all three EBNA promoters Cp, Wp, and Qp during both the latency and lytic cycles, the backsplicing of exon U was detected in all the examined type I and type III latency cells as well as cells harboring lytic reactivation.

LMP2 Locus

A total of eight vcircRNAs were derived from the LMP2 locus (Toptan et al., 2018; Ungerleider et al., 2019b). LMP2 is mainly a Type II/III latency protein that is expressed in a wide array of EBV associated B- and epithelial cell tumors where it likely contributes to the tumor phenotype (Yuan et al., 2006). Notably, the LMP2 protein is also expressed during the lytic stage, indicating a potential lytic function of these LMP2 products (Lu et al., 2006; Yuan et al., 2006; Concha et al., 2012). Furthermore, previous studies have shown that LMP2 locus undergoes extensive alternative splicing to generate various linear

TABLE 2 | Summary of currently identified circRNAs encoded by human oncogenic viruses and their biological effects.

Viruses	circRNAs	Parental gene	Cluster	Localization	Biological effects	Functional Validation	Sequencing method /algorithm	References
EBV	circEBNA _W1_C1	EBNA	EBNA latency locus	Nucleus	Potential non-coding regulatory function in Type III latency and lytic cycle	N/A	RNase R-seq/ ribodepletion -seq/find_circ	Ungerleider et al., 2018
	circEBNA _W2_C1	EBNA	EBNA latency locus	Nucleus	Potential non-coding regulatory function in Type III latency	N/A		
	circEBNA_U	EBNA	EBNA latency locus	N/A	Encodes potential peptides; likely functions in Type I/III latency and lytic cycle	N/A		
	circRPMS1 _E4_E3a	RPMS1/BART	RPMS1 locus	Nucleus	Likely regulates EBV miRNA biogenesis; likely functions in both latency and lytic cycle	N/A		
	circRPMS1 _E4_E2	RPMS1/BART	RPMS1 locus	Nucleus	Potential non-coding regulatory function; likely functions in both latency and lytic cycle	N/A		
	circRPMS1 _E7_E2	RPMS1/BART	RPMS1 locus	N/A	Lytic cycle-related function	N/A		
	circRPMS1 _E7_E3a	RPMS1/BART	RPMS1 locus	N/A	Lytic cycle-related function	N/A		
	circRPMS1 _E7_E3b	RPMS1/BART	RPMS1 locus	N/A	Lytic cycle-related function	N/A		
	circRPMS1 _E7_E5	RPMS1/BART	RPMS1 locus	N/A	Lytic cycle-related function	N/A		
	circRPMS1 _E7_E5.1	RPMS1/BART	RPMS1 locus	N/A	Lytic cycle-related function	N/A		
	circRPMS1 _E7_E5.2	RPMS1/BART	RPMS1 locus	N/A	Lytic cycle-related function	N/A		
	Other circRPMS1 variants	RPMS1/BART	RPMS1 locus	N/A	N/A	N/A		
	circLMP2 _E8_E2	LMP2	LMP2 locus	Nucleus/ Cytoplasm	Encodes potential LMP2A inhibitor; Type III latency/lytic cycle-related function	N/A		
	circLMP2 _E7_E2	LMP2	LMP2 locus	N/A	Lytic cycle-related function	N/A		
	circLMP2 _E5_E3	LMP2	LMP2 locus	N/A	Type III latency/lytic cycle-related function	N/A		
	Other circLMP2 variants	LMP2	LMP2 locus	N/A	N/A	N/A		
	circBHLF1	BHLF1	BHLF1 locus	Nucleus	Likely regulates lytic DNA replication; Encodes potential peptides in Type II/III latency and lytic cycle	N/A		
	circBHRF1	BHRF1	Other	N/A	N/A	N/A		
	circBART_1.1/ circRPMS1_E4 _E2_E3a_ I3a_E3b	RPMS1/BART	RPMS1 locus	Nucleus	Potential non-coding regulatory function	N/A	RNase R-seq/ CIRI2/ CIRCexplorer	Toptan et al., 2018
	circBART_1.2/ circRPMS1_E4 _E2_E3a_ E3b	RPMS1/BART	RPMS1 locus	Nucleus/ Cytoplasm	Potential non-coding regulatory function	N/A		
	circBART_2.1/ circRPMS1_E4 _E3a_I3a_ E3b	RPMS1/BART	RPMS1 locus	Nucleus	Potential non-coding regulatory function	N/A		
	circBART_2.2/ circRPMS1_	RPMS1/BART	RPMS1 locus	Nucleus/ Cytoplasm	Potential non-coding regulatory function	N/A		

(Continued)

TABLE 2 | Continued

Viruses	circRNAs	Parental gene	Cluster	Localization	Biological effects	Functional Validation	Sequencing method /algorithm	References
	E4_E3a_E3b circLMP2 circBHLF1	 LMP2 BHLF1	 LMP2 locus BHLF1 locus	 N/A N/A	 N/A N/A	 N/A N/A		
	circRPMS1/ circRPMS1_E4 _E2_E3	RPMS1/BART	RPMS1 locus	Nucleus/ Cytoplasm	Enhances tumor cell migration; likely function as a sponge for EBV-encoded miRNAs	Moderate evidence. Validated by RT-qPCR and wound healing assays.	Ribodepletion-seq/in-house algorithm	Huang et al., 2019
	circRPMS1	RPMS1/BART	RPMS1 locus	N/A	Inhibits tumor cell growth and EMT by sponging multiple cellular miRNAs including miR-31, 203, and 451; prognosis marker	Moderate evidence. Validated by cell proliferation, apoptosis, and invasion assays.	N/A	Liu Q. et al., 2019
	circLMP2A/ circLMP2 _E5_E3_E4	LMP2	LMP2 locus	Cytoplasm/ Nucleus	Induces cancer stemness by targeting the miR-3908/TRIM59/p53 signaling axis	Strong evidence. Validated by various molecular cellular biology methods using both in vitro cell model and in vivo animal model.	RNase R-seq/ in-house algorithm	Gong et al., 2020
KSHV	circ-vIRF4.IR/circ-vIRF4_E2_ E1_intron	vIRF4	vIRF4 locus	Nucleus	Potential non-coding regulatory function	N/A	RNase R-seq/ CIRI2/ CIRCexplorer	Toptan et al., 2018; Abere et al., 2020a
	circ-vIRF4/circ-vIRF4_E2_E1	vIRF4	vIRF4 locus	Cytoplasm	Loaded into new progeny virions; likely function as immediate-early gene products in newly infected cells	N/A		
	circPAN variants	PAN	PAN/K7.3 loci	Nucleus/ cytoplasm	Loaded into new progeny virions; likely function as immediate-early gene products in newly infected cells	N/A		
	circK7.3 variants	K7.3	PAN/K7.3 loci	Nucleus/ cytoplasm	Loaded into new progeny virions; likely function as immediate-early gene products in newly infected cells	N/A		
	circvIRF-4	vIRF4	vIRF4 locus	Nucleus/ cytoplasm	Potential Non-coding regulatory function in both latency and lytic cycle	N/A	RNase R-seq/ find_circ	Ungerleider et al., 2019a
	kcirc54	ORF34	Other	N/A	Dose-dependent regulation of viral RTA gene expression; regulation of cell growth	Moderate evidence. Validated by RT-digital PCR and cell viability assays.	RNase R-seq/ CIRC explorer2.3	Tagawa et al., 2018

(Continued)

TABLE 2 | Continued

Viruses	circRNAs	Parental gene	Cluster	Localization	Biological effects	Functional Validation	Sequencing method /algorithm	References
	kcirc55	ORF34/35/36	Other	N/A	Dose-dependent regulation of viral RTA gene expression; regulation of cell growth	Moderate evidence. Validated by RT-digital PCR and cell viability assays.		
	kcirc97	ORF60/61/62	Other	N/A	Dose-dependent regulation of viral RTA gene expression; regulation of cell growth	Moderate evidence. Validated by RT-digital PCR and cell viability assays.		
	kcirc3	ORF4/6	Other	N/A	N/A	N/A		
	kcirc29	PAN/K7	PAN/K7.3 loci	N/A	N/A	N/A		
	kcirc38	ORF21/22	Other	N/A	N/A	N/A		
	kcirc57	ORF34/35/36/37	Other	N/A	N/A	N/A		
HPV	circE7	E7	N/A	Cytoplasm/ Nucleus	Can be translated to the full-length E7 through m^6A-mediated cap-independent mechanism; regulate cell proliferation/oncogenesis	Strong evidence. Validated by various molecular cellular biology methods using both in vitro cell model and in vivo animal model.	ribodepletion-seq/polyA RNA-seq/vircircRNA	Zhao et al., 2019
MCV	circMCV _1622_861/ circMCV-T	ER loci	N/A	Cytoplasm	Forms a dynamic regulatory circuit with miR-M1 and linear T antigen transcripts to control MCV infection	Strong evidence. Validated by comprehensive molecular cellular biology methods using *in vitro* cell model.	RNase R-seq/CIRI2	Abere et al., 2020b
	circMCV_2955_861	ER loci	N/A	N/A	N/A	N/A		
	circMCV_3337_861	ER loci	N/A	N/A	N/A	N/A		
	circMCV_3913_3271	LR loci	N/A	N/A	N/A	N/A		
HBV	HBV_circ_1	pgRNA	N/A	N/A	Detected in the HCC cells and may be involved in oncogenesis	N/A	RNase R-seq with unknown detection algorithm	Zhu et al., 2020

N/A: Not applicable.

transcript isoforms during reactivation (Concha et al., 2012; O'Grady et al., 2014, 2016).

Among circLMP2s, high levels of circLMP2_E8_E2 were detected in reactivated Akata BL cells. This vcircRNA was generated by backsplicing of exon 8 to exon 2 of the LMP2 gene. Surprisingly, although Type III latency cells also constitutively express LMP2, circLMP2s were barely detected. It's postulated that certain tissue specific splicing factors are likely involved in the circLMP2 biogenesis.

BHLF1 Locus

circBHLF1 is derived from the intra-exonic backsplicing of the BHLF1 gene (Toptan et al., 2018; Ungerleider et al., 2018). Increasing evidence has indicated that BHLF1 can be expressed in both latency and lytic cycles and may work as a non-coding gene for various EBV strains (Lin et al., 2010, 2013; Yetming et al., 2020). The expression level of circBHLF1 is largely consistent with the isogenic linear transcript and it can be detected in most of the latency and reactivation conditions examined.

Regulation of EBV-Encoded circRNA Expression

Notably, although most of the vcircRNAs were encoded by the latency genes, many of these vcircRNAs can be detected in both latency and reactivation conditions. Nevertheless, higher levels of vcircRNAs were detected in the lytic reactivation. Liang et al. (2017) have shown that cell stress and associated depletion of spliceosomal components can increase production of circRNA by re-directing nascent RNAs into circRNA biogenesis pathways. Since reactivation elicits strong stress to the host transcription machinery, it's possible that EBV might utilize similar strategy to promote vcircRNA production. However, the level of the backsplicing of cellular genes is not induced during reactivation (Ungerleider et al., 2018). It suggests that the expression bias is likely due to virus-related mechanisms but not simply resulting from attenuated linear canonical splicing induced by the reactivation-related stress responses. Among the candidate viral factors regulating the biogenesis/transportation of these vcircRNAs, BMLF1 likely attracts the most attentions. BMLF is a viral lytic gene and it is actively involved in splicing, polyadenylation, RNA transport, and translation by interacting with various host factors including splicing regulators (Semmes et al., 1998; Hiriart et al., 2005; Sergeant et al., 2008; Juillard et al., 2009, 2012; Mure et al., 2018). However, whether BMLF1 can indeed regulate the biogenesis of these vcircRNAs remains to be explored in the future.

Function of EBV-Encoded circRNA Expression

RPMS1 Locus

Epstein-Barr virus circRNAs likely perform a wide spectrum of nuclear and cytoplasmic functions to facilitate viral pathogenesis. Given the universal expression of circRPMS1 in EBV-associated tumor tissues/cells as well as strong evolutionary conservation between EBV circRPMS1 and its rLCV homologue, it's reasonable to hypothesize that circRPMS1 may play an important role to facilitate EBV pathogenesis and associated tumor development (Ungerleider et al., 2019a).

Indeed, Liu Q. et al. (2019) found higher levels of circRPMS1 in the metastatic NPC tissues collected from patients with a shorter overall survival. Further, the authors provided both *in vitro* and *in vivo* evidence showing that circRPMS1 likely functions as key oncogene to facilitate NPC development. By sequestering/sponging a number of host miRNAs including miR-203, miR-31 and miR-451, circRPMS1 can promote proliferation, apoptosis-resistance, and epithelial-mesenchymal-transition (EMT) of the tumor cells and thus enhance the oncogenic processes (Liu Q. et al., 2019).

In another work, Huang et al. utilized computational approaches to identify a number of human and EBV miRNAs that may be directly sequestered by circRPMS1 (Huang et al., 2019). Some potential miRNA candidates have been shown to negatively regulate tumor development (e.g., hsa-miR-28-5p) or modulate EBV lytic cycles (e.g., ebv-miR-BART20-5p). Moreover, enhanced expression of circRPMS1 in a gastric cancer cell model significantly downregulated host miRNA candidates and increased cell motilities. This evidence supports an oncogenic role of circRPMS1.

Recently, Qiao et al. (2019) did a more comprehensive bioinformatics analysis of miRNAs candidates sponged by the EBV circRNAs including circRPMS1. They predicted that these targeted miRNAs are likely involved in herpesvirus infection, cell cycle regulation and various oncogenic pathways.

Meanwhile, since the backsplicing sites for circRPMS1_E4_E3a_I3a_E3b (circBART_2.1) and circRPMS1_E4_E3a_E3b (circBART_2.2) are in close proximity to the intron encoding EBV miRNAs, it's postulated that the backsplicing is likely to regulate these miRNA biosynthesis. Although circRPMS1 may still function as protein-coding RNAs in certain circumstances (e.g., in the *in vivo environment*), they are not associated with translated polysome fractions in the examined cells (Toptan et al., 2018).

EBNA Latency Locus

Given that both circEBNA_W1_C1 and circEBNA_W2_C1 are enriched in the nucleus, they are less likely to function as protein-coding transcripts. Further, the restricted expression of these vcircRNAs in Type III latency suggests that they may exhibit some latency-specific regulatory functions. For instance, they may be involved in the regulation of Cp activity and/or Cp-associated transcript diversity.

The evolutionary conservation of circEBNA_U between EBV and rLCV as well as similar expression of homologous rLCV_circEBNA_U in monkey lymphoma samples highly suggest a functional importance of this vcircRNA in viral pathogenesis (Ungerleider et al., 2019a). Interestingly, circEBNA_U contains an open reading frame (ORF) encoding a short 6-amino acid peptide. Moreover, ribosome profiling analysis showed possible translation initiation from this ORF (Bencun et al., 2018). With a potential IRES (internal ribosome entry site) translation initiation site, circEBNA_U may indeed encode some short peptides to regulate viral pathogenesis.

LMP2 Locus

Generally, LMP2 gene encodes two major isoforms, LMP2A and LMP2B. LMP2A is encoded by exons 1 to 9, and functions as a transmembrane signaling molecule (Longnecker and Kieff, 1990). In contrast, LMP2B is encoded by exons 2 to 9 (Longnecker and Kieff, 1990). Without the first exon encoding a cytoplasmic signaling domain, LMP2B mainly functions as a dominant-negative transmembrane inhibitor of LMP2A (Rovedo and Longnecker, 2007). Structure analysis shows that the circLMP2_E8_E2 likely carries all the coding sequence of the LMP2B. Since circLMP2_E8_E2 shows a significant cytoplasmic distribution, it's likely that this vcircRNA could function as a novel isoform of LMP2B to regulate LMP2A's function. In addition, the active expression of circLMP2_E8_E2 during the lytic cycle also indicates a potential role for this vcircRNA in reactivation.

Having analyzed the highly malignant SNU-4th cells isolated from the gastric cancer xenografts, Gong et al. provided evidence demonstrating that the EBV-encoded circLMP2A_E5_E3 likely functions as a miRNA sponge to induce and maintain cancer

stemness by suppressing the anti-tumor effects of the miR-3908/TRIM59/p53 signaling, which is likely responsible for the metastasis and poor prognosis of EBV-associated gastric cancer (Gong et al., 2020).

BHLF1 Locus

Cell fraction analysis shows that the circBHLF1 is mainly localized in the nucleus, indicating a nuclear function of this vcircRNA. Interestingly, previous studies have shown that BHLF1 linear transcript is associated with the lytic replication complex and promotes lytic viral DNA replication (Rennekamp and Lieberman, 2011). Thus, circBHLF1 may similarly interact with the proximal oriLyt DNA sequences and regulate viral DNA replication. The expression of circBHLF1 during the latency indicates that this vcircRNA may exhibit certain latency-related function as well.

circBHLF1 also carries an ORF encoding a putative 200 amino acid peptide. Evidence of translation initiation at this genomic location was reported previously (Bencun et al., 2018). However, it still remains unclear if the low abundance of circBHLF in the cytoplasm can produce this putative peptide, or if the product is functional.

KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

Kaposi's sarcoma-associated herpesvirus is also known as human herpesvirus 8 and is causatively associated with a number of human malignant diseases including Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD) (Chang et al., 1994; Cesarman et al., 1995, 1996; Soulier et al., 1995). As a human γ-herpesvirus, KSHV also has two distinct stages, latency and lytic phase in its life cycle. During the latency, relatively restricted sets of viral genes are expressed in order to minimize the mounted host adaptive immunity (Staskus et al., 1997; Katano et al., 2000; Parravicini et al., 2000; Hosseinipour et al., 2014). In the lytic cycle, more than 90 viral genes are expressed to facilitate the production of new infectious virions.

Like EBV, KSHV encodes its own non-coding linear RNAs to facilitate their needs for long-term persistence and transmission. At least 25 mature miRNAs have been identified in KSHV. This cluster of miRNAs is derived from a viral latency-associated genomic region and plays critical roles to support viral latency and associated oncogenesis (Cai et al., 2005; Pfeffer et al., 2005; Samols et al., 2005; Hansen et al., 2010; Lei et al., 2010a,b; Qin et al., 2010; Umbach and Cullen, 2010; Gottwein et al., 2011).

KSHV also encodes a number of lncRNAs including PAN (polyadenylated nuclear) RNA, K7.3, T3.0, T1.2, and ALT (antisense-to-latency transcript) (Staskus et al., 1997; Sarid et al., 1998; Wang et al., 2004; Taylor et al., 2005; Chandriani et al., 2010; Majerciak et al., 2013; Arias et al., 2014; Rossetto and Pari, 2014; Schifano et al., 2017). Most of these viral lncRNAs show increased expression during the lytic cycle and PAN RNA accounts for more than 80% of the viral transcripts during viral reactivation (Rossetto and Pari, 2014). Further, PAN RNA is also the most thoroughly studied KSHV lncRNA and it's involved in transcriptional and epigenetic regulation of host and viral gene expression (Rossetto and Pari, 2011, 2012; Rossetto et al., 2013). For instance, PAN facilitates late lytic gene expression in the nucleus and PAN may also modulate the host immune response to facilitate viral infection (Borah et al., 2011; Rossetto and Pari, 2011, 2014; Rossetto et al., 2013; Campbell et al., 2014a,b). Intriguingly, cytoplasmic PAN transcripts can be actively loaded into KSHV virions and likely function as immediate early RNA in the newly infected cells (Bechtel et al., 2005). K7.3 RNA is derived from the opposite strand to PAN. K7.3 RNA is also increased during lytic cycle and it's predicted to exhibit some non-coding function (Dresang et al., 2011; Schifano et al., 2017).

Kaposi's Sarcoma-Associated Herpesvirus Encoded circRNAs

Recently, several groups have reported that KSHV encoded a repertoire of circRNAs in virally-infected cell lines and clinical specimens including primary KSHV-associated tumor tissues and blood samples (Tagawa et al., 2018; Toptan et al., 2018; Ungerleider et al., 2019a; Abere et al., 2020a). These vcircRNAs are mainly derived from 2 genomic loci. In addition, a number of low-abundant vcircRNAs (such as kcirc3, kcirc38, kcirc54, kcirc55, kcirc57, and kcirc97) derived from several other genomic regions were also detected in certain cell model systems (Tagawa et al., 2018).

PAN/K7.3 Loci

More than 100 low-abundance vcircRNAs are derived from the PAN/K7.3 loci (Tagawa et al., 2018; Toptan et al., 2018; Ungerleider et al., 2019a; Abere et al., 2020a). These vcircRNAs can be further categorized into two distinct clusters: circPAN and circK7.3 based on their parental sense (PAN) as well as antisense (K7.3) transcripts, respectively. Although the level of individual vcircRNA within each cluster is low, the accumulated abundance of the circPAN and circK7.3 is very high and they become a major component of the KSHV circRNAome. Both circPAN and circK7.3 were detected in the analyzed PEL cell lines regardless of KSHV life cycle, as well as KSHV-associated tumor tissues (e.g., KS and MCD) and blood samples. Further, both circPAN and circK7.3 are distributed in both cytoplasm and nucleus.

vIRF Locus

circ-vIRF4s are derived from the vIRF4 (viral interferon regulatory factor 4)/K10 gene locus. The parental gene vIRF4 together with three other vIRFs namely vIRF1, vIRF2, and vIRF3 locate in a 10 kb genomic segment. The vIRF4 is homologous to cellular IRF4 and can inhibit the cellular IRF4 and it's also involved in cell cycle control and oncogenic process (Lee et al., 2009; Jacobs and Damania, 2011).

circ-vIRF4s are generated by backsplicing of exon 2 to exon 1. Two isoforms are observed. circ-vIRF4 is a 530 bp exonic circRNA, while circ-vIRF4.IR is a 632 bp Exon-Intron circRNA. Differential subcellular distributions were observed for the two circ-IRF4 isoforms. The intron retention form (circ-vIRF4.IR) is predominantly enriched in the nucleus. The exon-only form (circ-vIRF4) is mainly located in the cytoplasm. RNA

decay analysis showed that the half-life of circ-vIRF4 (∼5 h) is significantly longer than the one of linear vIRF4 mRNA (∼2 h). circ-vIRF4s can be detected in PEL cell lines, KSHV-associated tumor tissues (e.g., KS and MCD) and blood samples.

Regulation of KSHV-Encoded circRNA Expression

The overlapping nature of circPAN and circK7.3 makes the characterization work more challenging. None of those backsplicing junctions are utilized to splice the known linear RNA transcripts from the PAN/K7.3 loci. To date, the mechanism for such hypervariable and bidirectional synthesis of circPAN/K7.3 remains unclear. Notably, circPAN and circK7.3 can be detected in both latency and lytic reactivation. After lytic induction, the levels of circPAN and circK7.3 are increased in parallel with the linear PAN and K7.3 transcripts. Thus, the synthesis of circPAN/K7.3 is likely at least partially regulated by the transcription initiation of their parental genes.

circ-vIRF4s are constantly expressed in both latency and lytic cycle. Although the parental gene vIRF4 is induced after lytic induction, the levels of circ-vIRF4s are barely increased or even decreased in some cell models. In certain cell models (e.g., BCP-1 and BBG1), higher levels of circ-IRF4s were observed than the isogenic linear transcript. This indicates that the observed latent circ-vIRF4s are regulated by an independent mechanism compared to the biosynthesis of linear vIRF4 transcript. For instance, the expression of circ-vIRF4s may be regulated by an unrecognized latency promoter.

Function of KSHV-Encoded circRNAs

To date, the roles of these vcircRNAs in KSHV's pathogenesis are still largely unclear. However, given the important roles of the parental genes of these vcircRNAs (as discussed earlier) and their (constitutive) expression profiles, it's postulated that these non-polysome-associated vcircRNAs are most likely to function as non-coding regulatory RNAs.

Intriguingly, circPAN, circK7.3, and cytoplasmic circ-vIRF4 (but not the nucleus circ-vIRF4.IR) can be actively loaded into the KSHV infectious virions. It's hypothesized that these vcircRNAs, just like viral tegument proteins carried by the progeny virions (Sathish et al., 2012), may function as immediate-early gene products and host-immune modulators to help establish the infection in the newly infected cells.

Recently, Tagawa et al. showed that enhanced expression of some individual vcircRNAs such as kcirc54, kcirc55, and kcirc97 suppressed the transcription of viral lytic gene RTA and also induced an altered growth behaviors. This suggests a potential role of these vcircRNAs in KSHV pathogenesis (Tagawa et al., 2018).

HUMAN PAPILLOMAVIRUS

Human papillomaviruses are small non-enveloped, double-stranded DNA viruses (Brentjens et al., 2002; Roden and Stern, 2018). To date, more than 200 types of HPV have been identified and they show epitheliotropic and mainly infect mucosal and cutaneous epithelia (Roden and Stern, 2018). Based on the clinical consequence of the viral infection, HPV can be classified as low-risk HPVs (LR-HPVs) that generally cause benign lesions such as warts, as well as high-risk HPVs (HR-HPVs) that can transform the infected cells and promote cancer development (Roden and Stern, 2018). Among HR-HPVs, HPV16, and HPV18 are the most prevalent high-risk types and are etiologically associated with a large number of human cancers that occur in the cervical, anogenital, and head and neck regions. The recent development and implementation of anti-HPV vaccines successfully help to reduce new infection caused by several HR-HPV and LR-HPV types, but unfortunately do not have any effect on the existing persistent infection (Roden and Stern, 2018). Since the development of HPV-associated cancers is a chronic process, it is predicted that the HPV-associated malignancies will continuously cause significant medical problems in the next 50 years at least.

Human papillomavirus has a small ∼8 kb DNA genome which carries both the early (E) gene loci and late (L) gene loci (Brentjens et al., 2002). The E loci are regulated by the early-promoter and they encode proteins (E1–E7) required for viral replication. Meanwhile, the late-promoter-regulated L loci encode the structural proteins (L1–L2) for progeny virion assembly (Brentjens et al., 2002). HR-HPV E6 and E7 are the two most characterized viral oncogenes. They can inhibit major tumor suppressor proteins such as p53 and Rb and promote cancer development (Brentjens et al., 2002). So far, HPV has not been reported to encode any linear non-coding transcripts such as lncRNA and miRNA.

Human Papillomavirus Encoded circRNAs

Recently, a number of HPV-encoded circRNAs have been detected in HR-HPV-infected cell model systems and clinical cancer samples (Chamseddin et al., 2019; Zhao et al., 2019). These vcircRNAs are generated by backsplicing of various splice sites located in both the E and L loci. Among these vcircRNAs, the most abundant form is known as circE7 which accounts for ∼1–3% of total E7 transcript and it is first identified in the HPV16 infected cells and cancer tissues. Analogous circE7 has been detected in other types of HR-HPVs including HPV18, 31, 33, 35, 45, and 58. This 472 bp vcircRNA is generated by joining a downstream splice donor in E1 gene to an upstream splice acceptor in E6 gene. circE7 also carries the entire ORF of E7.

Regulation of HPV-Encoded circRNA Expression

Current evidence shows that the biogenesis of circE7 is likely partially regulated by its m^6A (N^6-methyladenosine) sites (Zhao et al., 2019). Indeed, mutation of circE7's m^6A sites leads to a decreased level of circE7 transcripts, indicating that these sites may be targeted by splicing factors required for circRNA biogenesis.

Function of HPV-Encoded circRNA Expression

The identification of multiple candidate miRNA binding sites on circE7 suggests that this vcircRNA may function as a miRNA sponge. Further, knockdown of HPV16 circE7 leads to an increased level of the linear E6/E7 transcripts, suggesting that circE7 may regulate the biogenesis of its isogenic transcripts by competing for their shared splice sites.

Besides its potential non-coding function, circE7 is likely to function as a protein-coding RNA, since it is enriched in the cytoplasm and also associated with translated polysome fractions. circE7 has been shown to produce a full length E7 protein in various cell models. The translation is apparently initiated through an m^6A (N^6-methyladenosine)-mediated cap-independent mechanism. HPV16 circ-E7-mediated expression of E7 protein has been shown to regulate cell proliferation, anchorage-independent cell growth, as well as oncogenesis in both *in vitro* cell models and *in vivo* tumor xenografts. Further, the expression of circ-E7 in virally infected non-cancer cells indicates that this vcircRNA may also play a role in viral infection cycle.

MERKEL CELL POLYOMAVIRUS

Merkel cell polyomavirus is causally associated with a majority of the Merkel cell carcinoma, an aggressive skin cancer mainly affecting elderly and immunocompromised individuals (Feng et al., 2008; Shuda et al., 2008). MCV is a nonenveloped virus with a circular double-stranded DNA genome and belongs to the human polyomavirus family (Feng et al., 2008; Shuda et al., 2008). In the infected cells, the MCV genome can remain either as a free episomal DNA undergoing active replication, or as a replication-deficient form after integrating into the host genome (Feng et al., 2008; Shuda et al., 2008). It is believed that genome integration plays a critical role in MCV-mediated oncogenesis (Feng et al., 2008; Shuda et al., 2008). Its relatively small DNA genome can be simply divided into two gene loci, the early region (ER) and late region (LR). Generally, the ER-encoded gene products such as large T antigen (LT) are involved in the regulation of viral replication and oncogenesis. For instance, the LT can directly target tumor suppressor protein Rb. Meanwhile, it can bind to the viral origin of replication and initiate viral genome replication with its intrinsic helicase/ATPase activity (Feng et al., 2008; Shuda et al., 2008). In contrast, the LR loci encode viral structural proteins such as VP1 and VP2 required for new virion production (Tolstov et al., 2009).

Besides viral protein products, MCV also encodes two miRNAs, namely MCV-miR-M1-5p and MCV-miR-M1-3p, which are derived from the pri-miRNA transcript synthesized from the negative strand of the ER (Seo et al., 2009). These 22 nucleotide viral miRNAs have been shown to inhibit MCV lytic replication by knocking-down the LT transcripts in order to facilitate maintenance of the episomal genome (Seo et al., 2009; Theiss et al., 2015).

Merkel Cell Polyomavirus Encoded circRNAs

Recently, MCV has been shown to encode four circRNAs (Abere et al., 2020b). Among them, three vcircRNA are derived from the ER loci and one is encoded by the LR loci. circMCV-T (circMCV_1622_861) is the most abundant one and accounts for more than 70% of the total vcircRNA population. circMCV-T is a 762 bp vcircRNA carrying the entire exon II of the T antigen. It's generated by backsplicing of the downstream 5′ splice donor (nt 1622) to the upstream 3′ splice acceptor (nt 861). These splice sites are also required for the splicing of all the linear T antigen isoforms including LT, small T antigen (sT), 57,000-molecular-weight T antigen (57 kT), and alternative LT open reading frame (ALTO) transcripts. circMCV_2955_861 and circMCV_3337_861 use the same 3′ splice acceptor sites but different 5′ splice donor sites at nt 2955 and 3337, respectively. circMCV_3913_3271 shows lowest abundance and is derived from the LR loci. To date, among these vcircRNAs, only the most abundant circMCV-T was further characterized.

Regulation of MCV-Encoded circRNA Expression

The circMCV-T transcripts are detected mainly in the cells harboring actively replicating episomal viral genomes, but rarely in the cells carrying more dormant integrated viral genome. This suggests that the biogenesis of circMCV-T is at least partially regulated by the transcription activity of its parental ER loci.

Function of MCV-Encoded circRNAs

A circMCV-T homologue, namely circRatPyV2_4112_4468 was recently detected in the RatPyV2 infected cells (Abere et al., 2020b). The evolution conservation of circMCV-T further indicates a functional significance of this RNA molecule. Increasing evidence has shown that the MCV-encoded transcripts including circMCV-T, miR-M1, and linear T antigen transcripts form a dynamic regulatory circuit to control MCV's life cycle. Due to a 100% sequence complementarity between miR-M1 and linear T antigen mRNAs, the miRNA-mRNA interaction can knock down linear T antigen transcripts and inhibit viral DNA replication. The presence of the new player circMCV-T further increases the complexity of the network. Due to a 100% sequence complementarity between miR-M1 and circMCV-T, the reciprocal interaction between circMCV-T and miR-M1 can lead to two scenarios. In the first scenario, miR-M1 can decrease the stability of circMCV-T by linearizing it and thus reduce the genome replication. In the second, circMCV-T can increase the level of linear T antigen transcripts and facilitate viral replication by sequestering miR-M1. Hence, the overall effects will be determined by the relatively amount of miR-M1 and circMCV-T: a condition of high circMCV-T and low miR-M1 will favor active MCV replication. Conversely, a low circMCV-T and high miR-M1 condition will suppress active viral replication and promote a long-term persistent infection.

Besides the non-coding function, the circMCV-T transcript may potentially function as a protein-coding RNA. The in situ staining data show that circMCV-T is predominantly located in

the cytoplasm. It also carries the entire exon 2 of T-antigen as well as the start codon for the ALTO ORF. However, some data show that circMCV-T transcripts are not associated with any translated polysomes in certain cell models.

HEPATITIS B VIRUS

Hepatitis B virus is a small oncogenic human virus closely associated with liver cirrhosis and HCC (Torresi et al., 2019). HBV has a 3.2 kb partially double-stranded relaxed circular DNA (rcDNA) genome. In the infected cells, a covalently closed circular DNA (cccDNA) genome is formed and remains as a persistent episomal genome. The cccDNA is used as templates to generate key transcripts including the pregenomic RNA (pgRNA) transcript for viral replication.

Hepatitis B Virus Encoded circRNA

An HBV-encoded circRNA has been recently identified in both HBV-infected cell models and HCC tissues (Sekiba et al., 2018; Zhu et al., 2020). Zhu et al. (2020) has shown that this 2.5 kb vcircRNA, namely HBV_circ_1 is derived by the intronless pgRNA. Surprisingly, instead of utilizing the backsplicing, it is believed that this vcircRNA is generated by a mechanism involving the homologous recombination of the inverted repeat sequences at both 3′ and 5′ ends of the pgRNA (Zhu et al., 2020). In addition, HBV_circ_1 is predominantly located in the cytoplasm (Zhu et al., 2020).

Regulation of HBV-Encoded circRNA Expression and Potential Function

Sekiba et al. (2018) showed that knockdown of an RBP DXH9 increases the levels of HBV_circ_1. It is proposed that the expression of HBV_circ_1 can be negatively regulated by the direct interaction between DHX9 and the inverted repeat regions of the pgRNA. To date, the biological function of HBV_circ_1 remains unclear.

POTENTIAL CLINICAL APPLICATION OF vcircRNAs

To date, detection of viral genomic material has been widely implemented in the clinical practice. For instance, circulating EBV DNA has been used as a good biomarker for the diagnosis and prognosis of EBV-associated NPC (Lo et al., 1999; Leung et al., 2006; Chan et al., 2017). Recently, circRNAs have also received increasing attention for its potential clinical applications. These highly stable vcircRNAs that exist in various types of body fluids and tissues may serve as good diagnostic and prognostic markers for many virally associated diseases.

Current evidence showed that vcircRNAs might be superior to the current RNA diagnostic marker. For EBV, EBERs are used as a gold standard for diagnosis of EBV infection (Ambinder and Mann, 1994). However, data showed that circRPMS1 were detected in certain PTLD tissues carrying reasonable number of EBV genomes, where EBER cannot be detected, indicating potential false-negative results coming from the EBER diagnostic test (Toptan et al., 2018). For KSHV, circPAN/K7.3 and circvIRF4 showed a higher detection rate than the linear viral transcript LANA (latency-associated nuclear antigen) in the analyzed primary KS tumor and associated blood samples (Abere et al., 2020a). Lastly, in the case of HPV, PCR-based detection of HR-HPV has been used for cervical cancer screening. The current data show that circE7 can be detected in fixed archive tissue samples as well (Chamseddin et al., 2019). It is warranted to investigate if circE7 may serve as a promising biomarker for HPV infection and HPV-related cancers.

CHALLENGES AND FUTURE DIRECTIONS

Due to the recent development of circRNA enrichment and high-throughput detection technologies, we are now in a rapidly growing era of vcircRNA research. Numerous novel vcircRNAs have been identified in human oncogenic viruses, and they are no longer viewed as insignificant splicing noise. Instead, vcircRNAs are now seen for what they are: an important new class of regulatory molecules in viral infection cycle and associated malignant diseases. The addition of this new member has created an entirely new layer of complexity of the viral pathogenic mechanism.

Nevertheless, the vcircRNA research is in its nascent stage where many puzzles remain to be solved. Currently, the underlying mechanism for the biogenesis of vcircRNAs is still largely unclear. It's postulated that, in addition to the general splicing machinery, both tissue-specific host factors and viral factors are likely to be involved in the formation of vcircRNAs. For instance, EBV circLMP2_E8_E2 can be detected in reactivated BL cells, but not in the type III latency cell lines that constitutively express parental linear LMP2A transcripts. It indicates that some tissue-specific factors may regulate the backsplicing by targeting the *cis* regulatory elements in the primary LMP2 transcript (Ungerleider et al., 2018). Meanwhile, EBV viral lytic gene BMLF1 is known to play critical roles in the maturation of viral linear transcripts and likely contribute to the backsplicing process. Future research is warranted to elucidate the detailed mechanism of vcircRNA synthesis.

Notably, within the family of human oncogenic viruses, it is still unclear if HCV or human T-lymphotropic virus (human T-cell leukemia virus type 1 or HTLV-1) encodes any vcircRNAs. Future research is surely warranted to investigate if any vcircRNAs are present in the HCV or HTLV-1-positive biospecimen. The RNase R-sequencing will be an ideal approach to evaluate the vcircRNAome in a high-throughput and unbiased manner.

Among all the human oncogenic viruses investigated so far, herpesviruses are undoubtedly the largest producer of vcircRNAs and account for >85% of the identified vcircRNAs. This expression bias may be partially attributable to herpesviruses' unique bi-phasic life cycles: they can exist in both latent and lytic phases. Particularly during the latency, to escape the host immune surveillance, herpesviruses cannot express

antigenic viral proteins, while they still need to regulate themselves and host environment. Hence, expression of non-coding RNAs which lack immunogenicity is likely an ideal strategy for the viruses to regulate themselves and the host environment when an immunologically transparent state is required. Increasing evidence has shown that vcircRNAs are less immunogenic, compared to linear transcripts. Chen et al. reported that unmodified exogenous circRNAs (without any nucleoside modifications) are recognized as "nonself" RNAs by the cellular sensors such as RIG-I and thus trigger the innate immune signaling. However, the circRNAs spliced by endogenous human spliceosomes are viewed as "self" RNAs and lose the immunogenicity, due to the N^6-methyladenosine (m^6A) modification during the biogenesis (Chen Y.G. et al., 2017; Chen et al., 2019). Based on the high prevalence of m^6A modification of viral transcripts and evidence that vcircRNAs are largely generated by the same host splicesomes used to synthesize "self" circRNAs, it is reasonably to believe that vcircRNAs are non-immunogenic (Wu F. et al., 2019; Lu et al., 2020). Notably, Wesselhoeft et al. recently reported a discordant observation that immunogenicity of circRNA is independent of m^6A modification. They found that unmodified exogenous circRNAs are also non-immunogenic and can avoid antiviral defense induction upon cellular entry by dodging immune sensors such as RIG-I and Toll-like receptor (Wesselhoeft et al., 2019). Although this new discovery still supports the notion that vcircRNAs are non-immunogenic, more efforts are warranted to investigate the discrepant data on the mechanism of immunoevasion of foreign circRNAs.

To date, only a handful of identified vcircRNAs are functionally investigated. A majority of these characterized vcircRNAs are enriched in the cytoplasm and are proposed to function as either miRNA sponges or protein-coding transcripts to regulate viral pathogenesis. These conclusions are largely based on the data from the *in vitro* cell models. However, the functional impacts of the large number of nuclear vcircRNAs have not yet been reported. These nuclear vcircRNAs such as EBV circRPMS1_E4_E2_E3a_I3a_E3b (circBART_1.1) and circRPMS1_E4_E3a_I3a_E3b (circBART_2.1) generally carry retained-introns from their parental genes. Due to their high abundance and nuclear distribution, they may play important regulatory roles such as modulating the levels of their isogenic transcripts by competing the splice sites and limited splicing factors. Further investigation is surely warranted.

The detailed transcript structures of these vcircRNA candidates are largely unclear. The size and sequence of most of these vcircRNAs are predicted simply based on their backsplicing sites. In-depth sequencing-based assembly and validation of the vcircRNAs is surely warranted, which is essential for the subsequent characterization work to understand the biogenesis and function of each vcircRNA isoform. It will be interesting to see if some new computation pipelines designed for reconstruction of full-length circRNA can be successfully implemented in vcircRNA research (Wu J. et al., 2019; Zheng et al., 2019).

To date, all the reported vcircRNAomes of these human oncogenic viruses are generated by RNA-seq analyses of bulk cell cultures and/or clinical tissues. Due to the inevitable heterogeneity of tissue/cell culture, the vcircRNAomes are likely different at the single-cell level. Thus, the expression and function of these vcircRNAs might vary among single cells. Implementation of single-cell sequencing technologies which are compatible with circRNAs might help us better decipher the biogenesis mechanism and function of vcircRNAs (Fan et al., 2015).

Our understanding of the biogenesis mechanism and function of these vcircRNAs are largely based on the data derived from *in vitro* cell cultures. These data need to be further validated and examined in more physiological relevant *in vivo* model systems such as animal models and clinical biospecimens.

The altered expression of vcircRNAs in infected host cells and diseased tissues as well as the long half-life of vcircRNAs make them potential biomarkers for clinical diagnosis of viral infection and prognosis of disease outcomes. Some of the oncogenic vcircRNAs such as circLMP2A might serve as potential targets for precision therapy. Given the successful implementation of mRNA-based vaccines to prevent SARS-CoV-2 infection, future highly stable circRNA-based vaccines might become a new tool to better prevent human oncogenic virus infection and related diseases.

The discoveries of viral miRNAs and lncRNAs and their roles have dramatically increased our knowledge of how oncogenic viruses interact with hosts and facilitate cancer development. Just like in the early years of viral miRNA and lncRNA research, the vcircRNA research has now started gaining increased attention and becoming an important topic in the viral oncology field. With further improvement of the circRNA analytical techniques, we expect that more vcircRNAs will be identified and successfully characterized in the coming years. These developments will help us better understand the roles of human oncogenic viruses in their associated cancers and improve patient prognosis in the fight against these deadly diseases.

AUTHOR CONTRIBUTIONS

ZL, JA, DB, and RA contributed to the conceptualization, drafting, and revision of the manuscript. AN, JK, and LL contributed to the conceptualization and manuscript revision. All authors contributed to the article and approved the submitted version.

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