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Involvement and Roles of Long Noncoding RNAs in the Molecular Mechanisms of Emerging and Reemerging Viral Infections

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ABBREVIATIONS

AFP alpha-fetoprotein
ATF2 activating transcription factor 2
CHC chronic HCV infection
EV71 enterovirus 71
HBV hepatitis B virus
HCC hepatocellular carcinoma
HDV hepatitis D virus
HEV hepatitis E
HIV human immunodeficiency virus
IAV influenza A virus

IL interleukine
IRG immunity-related GTPase
ISGs interferon-stimulated genes
JEV Japanese encephalitis virus
LncRNAs long noncoding RNAs
MDM monocyte-derived macrophages
MiRNAs microRNAs
NcRNAs noncoding RNAs
NGS next-generation sequencing
NI nonidentified
PCR polymerase chain reaction
PiRNAs Piwi RNAs
PRC2 polycomb repressive complex 2
qPCR quantitative PCR
qRT-PCR quantitative real-time PCR
RNA-Seq RNA sequencing
SARS-CoV severe acute respiratory syndrome coronavirus
siRNAs small interfering RNAs

INTRODUCTION

Genome analysis, especially using the next-generation sequencing approaches, has revealed that only 2% of the human genome is composed of protein-coding genes, while the largest part that was considered dark matter transcribed into diverse RNAs with no protein-coding capacity (Takayama and Inoue, 2015). Noncoding RNAs (ncRNAs) are classified based on their length; there are short ncRNAs of less than 200 nucleotides and long ncRNAs (lncRNA) larger than 200 nucleotides. Further division of short differentiate Piwi-interacting RNAs, small interfering RNAs, and microRNAs (miRNAs) (Wang et al., 2013).

Transcription of lncRNAs generally involves RNA polymerase II or III. Notably, these transcripts present similarities with mRNAs such as the 5'cap and the polyadenylated tail in some cases at the 3'end. Nevertheless, lncRNAs are less abundant in the cell compared to mRNAs and their expression is more specific to the cell, tissue, organ type, and to the developmental stage (Nguyen and Carninci, 2016). According to their position relative to the neighboring protein-coding gene, lncRNAs are classified as sense, antisense, bidirectional, intronic, or intergenic. In most cases, lncRNAs regulate expression of neighboring genes with whom they probably share the same enhancers, in *Cis*, or in *Trans*, if the genes are distant (Laurent et al., 2015).

Recent studies have revealed different roles of lncRNAs, and they are now known for their important functions within the cell, as they are involved in the regulation of cell physiology through epigenetic regulation of gene expression, either by modifying the chromatin state or

regulating transcriptional and posttranscriptional events. Thus lncRNAs are incriminated in several pathologies, such as Alzheimer, cancer, and hepatocellular carcinoma (HCC) as reviewed recently by DiStefano (Hasegawa and Nakagawa, 2015; Liu and Ding, 2017; DiStefano, 2018; Josipovic et al., 2018).

Comparing to available data on lncRNAs in malignant diseases, discoveries on the involvement of these transcripts in emerging viral pathologies are in their infancy. Nevertheless, accumulating evidence from transcriptomic studies showed different expression patterns of lncRNAs during viral infections and suggests regulatory roles of these molecules (Peng et al., 2010; Zhang et al., 2016a,b; Fan et al., 2017; Wang et al., 2017). To assess the mechanism underlying this regulation, coexpression and pathway analysis studies have been carried. Actually, they revealed the correlation of lncRNAs with mRNAs and identified lncRNAs–mRNAs partners that may interfere together in many signaling pathways, related to viral invasion and antiviral immune response (Ma et al., 2017b).

In fact, lncRNAs play roles in controlling the virus–host interface. After viral entry, lncRNAs regulate genes of immune response responsible for interferon (INF) production in order to transduce signals to the immune cells for activation (Ouyang et al., 2014; Tang et al., 2017). However, viruses hijack these mechanisms by interfering with cellular lncRNAs, along with coding genes and proteins. Moreover, viral pathogens dispose of their own lncRNAs within their genome and they use them to create favorable environment to their replication and progeny (Saayman et al., 2014). In this chapter, we discuss the molecular mechanisms involving lncRNAs in viral–host interface, with an emphasis on examples of emerging viral pathogens.

LONG NONCODING RNAS IN VIRUS BIOLOGY: EXAMPLES OF EMERGING VIRAL PATHOGENS

Viral pathogens initiate their infectious process when sufficient viral particles are available in contact with accessible, susceptible, and permissive cells. Giving that, viruses are obligate parasites that dispose of small genomes, they need to hijack and manipulate their host molecular resources to synthesize the essential proteins required for completing their life cycle and replicate their genome to produce new viral particles. Using noncoding transcripts such as miRNAs and lncRNAs is among the newly discovered strategies of viruses that are currently the subject of extensive studies. However, there has been few published data to understand the link between lncRNAs and emerging viral infections. The main recent findings are summarized in [Table 6.1](#).

TABLE 6.1 Main Studies on Long Noncoding RNAs (lncRNAs) in Emerging Viral Pathogens

Virus	lncRNAs	Author/ year	Detection and quantification technique	Main findings
Respiratory viruses	NI	Peng et al. (2010)	<ul style="list-style-type: none"> • NGS • Microarray • qRT-PCR 	<ul style="list-style-type: none"> • Discovery of the differential expression of lncRNAs during viral infections in SARS-CoV and IAV-infected cells • lncRNAs are possibly involved in regulating the host response
	NI	Josset et al. (2014)	RNA-Seq	<ul style="list-style-type: none"> • Differentially expressed lncRNAs were coexpressed with differentially expressed mRNAs • <i>Cis</i>-Regulatory lncRNAs were correlated with neighboring coding genes • 5 lncRNAs were predicted to be ISGs
	VIN	Winterling et al. (2014)	<ul style="list-style-type: none"> • Microarray • qRT-PCR 	<ul style="list-style-type: none"> • Different IAV strains (H1N1, H3N2, and H7N7) induce the expression of VIN • VIN expression constitutes a specific response to some viral infections and contributes to the virulence of the responsible viral pathogens

(Continued)

TABLE 6.1 (Continued)

Virus	LncRNAs	Author/ year	Detection and quantification technique	Main findings
HIV	NEAT1	Zhang et al. (2013)	<ul style="list-style-type: none"> • qPCR array • qRT-PCR 	<ul style="list-style-type: none"> • NEAT1 expression levels are enhanced following HIV-1 infection • HIV-1 production is mediated by depletion of NEAT1 • NEAT1 induces the production of paraspeckle bodies as a mechanism of HIV-1 viral replication
	HIV- encoded lncRNA	Saayman et al. (2014)	qRT-PCR	HIV gene expression is regulated by HIV-encoded lncRNA through an epigenetic mechanism
	NRON	Imam et al. (2015)	<ul style="list-style-type: none"> • PCR array • NGS • Semiquantitative and qRT-PCR 	NRON is regulated by HIV-1 accessory proteins Nef and Vpu and modulates HIV-1 replication through NFAT-mediated pathway
		Li et al. (2016)	qRT-PCR	<ul style="list-style-type: none"> • NRON participates in maintaining HIV-1 latency • NRON represses HIV-1 replication and transcription through Tat protein degradation
	NI	Trypsteen et al. (2016)	<ul style="list-style-type: none"> • Microarray • qRT-PCR 	Five differentially expressed lncRNAs–mRNAs were involved in HIV pathogenesis

(Continued)

TABLE 6.1 (Continued)

Virus	LncRNAs	Author/ year	Detection and quantification technique	Main findings
Herpesviruses	ASP RNA	Zapata et al. (2017)	<ul style="list-style-type: none"> • qRT-PCR 	<ul style="list-style-type: none"> • Expression of ASP RNA suppresses viral replication • Expression of ASP RNA promotes the establishment and maintenance of HIV-1 latency through its interaction with the PRC2 complex
	LNC 173	Postler et al. (2017)	<ul style="list-style-type: none"> • In silico analysis • qRT-PCR 	LINC00173 is upregulated during infection with HIV
	PAN	Borah et al. (2011)	Immunofluorescence <ul style="list-style-type: none"> • RNA-protein pulldown assays • IP <ul style="list-style-type: none"> • ChIRP-Seq • ELISA 	PAN RNA was also found to interact in the nucleus with PABPC1 <ul style="list-style-type: none"> • PABPC1 suppresses PAN RNA expression • ORF57 targets PABPC1 to enhance PAN RNA accumulation • PAN can bind promoters of viral genes and activate their expression to induce the removal of the H3K27me3 mark for activation • PAN acts as a repressor by interacting with components of the repressive PRC2 complex

(Continued)

TABLE 6.1 (Continued)

Virus	LncRNAs	Author/ year	Detection and quantification technique	Main findings
	ALT	Campbell et al. (2014)	<ul style="list-style-type: none"> • RT-PCR • RNA-Seq 	<ul style="list-style-type: none"> • PAN RNA promotes LANA-episome disassociation during KSHV reactivation <p>ALT RNA is identified as a bona fide lncRNA of KSHV</p>
	NI	O'Grady et al. (2013)	RNA-Seq	<ul style="list-style-type: none"> • During reactivation of EBV infection, late antisense transcripts are overexpressed in the nucleus • The process of viral production is highly regulated by noncoding transcripts
	NAG7/ LINC00312	Zhang et al. (2013)	Microarray	<ul style="list-style-type: none"> • NAG 7 is downregulated in NPC tissues compared to normal controls • NAG 7 expression is negatively correlated to tumor size and positively correlated to lymph node metastasis
	BART lncRNAs	Marquitz et al. (2015)	RNA-Seq	First report of the function of spliced and polyadenylated transcripts of BARTs genes in regulating cellular growth of EBV-induced epithelial cancer

(Continued)

TABLE 6.1 (Continued)

Virus	LncRNAs	Author/ year	Detection and quantification technique	Main findings
EV71	-oriPtL- oriPtR	Cao et al. (2015)	RNA-Seq	The latency origin of replication oriP encodes two late noncoding transcripts that interact with paraspeckles in the innate immune response to viruses and promote lytic gene expression of EBV
	LOC553103	He et al. (2016)	qRT-PCR	<ul style="list-style-type: none"> • LOC553103 promotes EBV-associated cancer metastasis and invasion • EBV-miR-BART6-3p suppresses metastasis through regulation of LOC553103
	H19		<ul style="list-style-type: none"> • PCR Array • qRT-PCR 	<ul style="list-style-type: none"> • H19 is highly expressed in EBV-transformed LCL • H19 is present in LCL exosomes
	SNHG8	Huang et al. (2016)	<ul style="list-style-type: none"> • RT-PCR • RNA-Seq 	SNHG8 regulates genes and EBV proteins, which modulate gastric cancer progression
	NI	Yin et al. (2013)	<ul style="list-style-type: none"> • Microarray • qRT-PCR 	<ul style="list-style-type: none"> • LncRNAs are aberrantly expressed in EV71-infected cells • LncRNAs regulate host–EV71 interactions

(Continued)

TABLE 6.1 (Continued)

Virus	LncRNAs	Author/ year	Detection and quantification technique	Main findings
HEV	BISPR	Paliwal et al. (2017)	<ul style="list-style-type: none"> • RNA-Seq • qRT-PCR 	BISPR regulates viral replication through modulating BST2 expression levels

EBV, Epstein–Barr virus; *EV*, enterovirus; *HEV*, hepatitis E virus; *HIV*, human immunodeficiency virus; *IAV*, influenza A virus; *IP*, immunoprecipitation; *ISGs*, interferon-stimulated genes; *KSHV*, Kaposi’s sarcoma-associated herpesvirus; *LANA*, latency-associated nuclear antigen; *LCL*, lymphoblastoid cell line; *NFAT*, nuclear factor of activated T cells; *NGS*, next-generation sequencing; *NI*, nonidentified; *NPC*, nasopharyngeal carcinoma; *NRON*, noncoding repressor of NFAT; *PABPC1*, poly(A)-binding protein C1; *PCR*, polymerase chain reaction; *qPCR*, quantitative PCR; *qRT-PCR*, quantitative real-time PCR; *RNA-Seq*, RNA sequencing; *SARS-CoV*, severe acute respiratory syndrome coronavirus; *VIN*, viral-induced noncoding.

LONG NONCODING RNAs CONTRIBUTE TO VIRAL PATHOGENICITY IN RESPIRATORY DISEASES

Previously, [Peng et al. \(2010\)](#) investigated lncRNAs as a response signature to viral infections using whole-transcriptome analysis of the host response to severe acute respiratory syndrome coronavirus (SARS-CoV). This study showed a differential expression of lncRNAs during SARS-CoV infection, which suggests an important role as new regulators of viral infections ([Peng et al., 2010](#)). Later, [Josset et al.](#) performed a transcriptional analysis of mouse SARS-CoV and influenza A virus (IAV) infected lungs and found 5329 differentially expressed lncRNAs. Importantly, some of these lncRNAs were found to be associated with nearby coding genes. In addition, these lncRNAs were predicted and validated to be stimulated by INF production, and therefore, they may play a key role in viral pathogenesis ([Josset et al., 2014](#)). Using microarray systems, [Winterling et al.](#) identified 42 differentially expressed lncRNAs in response to IAV infection. Among studied lncRNAs, vesicular stomatitis virus and H1N1, H3N2, H7N7 strains of IAV, induced viral-induced noncoding RNA (VIN RNA). Moreover, RNA interference knockdown of VIN RNA repressed the protein synthesis and replication of IAV ([Winterling et al. 2014](#)). These findings suggest a common mechanism of respiratory viruses’ pathogenicity involving lncRNAs.

LONG NONCODING RNAS REGULATE HUMAN IMMUNODEFICIENCY VIRUS REPLICATION AND MAINTAIN ITS LATENCY

In human immunodeficiency virus (HIV), [Zhang et al. \(2013\)](#) characterized for the first time the lncRNA NEAT1 for its role in HIV-1 biology. Among 83 studied lncRNAs, NEAT1 was one of many lncRNAs that have been enhanced in expression upon HIV-1 infection ([Zhang et al., 2013](#)). Furthermore, NEAT1 has been shown to posttranscriptionally regulate HIV-1 replication through inducing paraspeckle bodies expression and the regulation of nucleus-to-cytoplasm export of Rev-dependent instability element (INS)-containing HIV-1 mRNAs ([Zhang et al., 2013](#)). In addition, [Saayman et al. \(2014\)](#) studied an HIV-encoded lncRNA that has been previously discovered and showed that this lncRNA binds to gene promoters and suggests its function as a negative epigenetic regulator of viral transcription and a promoter of HIV latency. NRON [noncoding repressor of NFAT (nuclear factor of activated T cells)] is another lncRNA that has been aberrantly expressed following the infection with HIV-1 and that it regulates HIV-1 replication through modulating NFAT activity ([Imam et al., 2015](#)). Moreover, HIV accessory protein, including Nef, which is an early HIV-1 protein, reduced NRON levels, resulting to viral replication, while the late protein Vpu increased its levels and thus promote HIV viral latency ([Imam et al., 2015](#)). Another mechanism of NRON-mediated latency of HIV was proposed by [Li et al. \(2016\)](#); they suggest that NRON is involved in inhibiting viral transcription through directing tat protein to the ubiquitin/proteasome components (CUL4B and PSMD11) resulting in its degradation. Several other lncRNAs have been reported for their putative roles in regulating HIV viral life cycle. To study the different expression patterns of lncRNA during viral life cycle, [Trypsteen et al. \(2016\)](#) performed a transcriptome profiling during HIV integration, reverse transcription, and HIV particle production. They also investigated the roles of these lncRNAs and found their involvement in different pathways of cell cycle regulation including responses to DNA damage, apoptosis, and proteasomal and ubiquitination pathways ([Trypsteen et al., 2016](#)). In addition, several lncRNAs have been reported to regulate gene expression through their interaction with chromatin modifying complexes, especially the polycomb repressor complex 2 (PRC2) that directs methylation marks to the promoters of targeted genes. Similarly, lncRNA ASP RNA was reported to interact with PRC2 at the HIV-1 5'LTR region and results in H3K27me3 mark accumulation ([Zapata et al., 2017](#)). Along with these findings, ASP RNA reduced RNA polymerase II expression ([Zapata et al., 2017](#)). As a result, HIV-1 replication is repressed and its latency is established and maintained ([Zapata et al., 2017](#)). More recently, [Postlera et al.](#) used a metaanalytic approach to investigate

differentially expressed lncRNAs during HIV-1 infection. One of the identified lncRNAs was proposed to be a regulator of cytokines regulation in T cells (Postler et al., 2017).

LONG NONCODING RNAS IN HERPESVIRUSES LATENT TO LYTIC CYCLE TRANSITION

Herpesviruses infect a wide range of organisms including plants and mammals. Mammalian Herpesviruses undergo two life cycles, latency is the phase where the virus does not produce any viral particles while lytic phases is when the virus reactivates and replicate its genome to produce new progeny. The most studies on herpesviruses and lncRNAs have been focused on Kaposi's sarcoma-associated herpesvirus (KSHV) and Epstein–Bar virus (EBV).

LONG NONCODING RNAS REGULATE THE TRANSITION FROM LATENT TO LYTIC PHASE IN KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS INFECTION

KSHV is associated with several diseases such as AIDS-related malignancy named Kaposi's sarcoma, KSHV inflammatory cytokine syndrome, and lymphoproliferative disorders primary effusion lymphoma (PEL), Multicentric Castleman's Disease and PEL (Borah et al., 2011; Conrad, 2016). During KSHV infection, a 1.1 kb long ncRNA PAN is produced in the early stages and accumulates in the nucleus. In fact, PAN RNA expression represents 80% of the total polyadenylated transcripts produced in the lytic phase by both infected cells and KSHV, which suggest an important role in latent to lytic phase transition of the virus (Borah et al., 2012). KSHV recruit several molecules in order to activate the expression of its own transcripts and the shutdown of host RNAs expression; these include ORF50 and ORF57 proteins that modulate viral gene expression and RNA processing. ORF57 interacts with PAN RNA and enhances its accumulation. ORF57 is also found to maintain PAN RNA stability in the absence of ENE elements through binding to a 9 nt motif contained in an element called MRE (Mta responsive element) located at the 5' region of PAN. In addition, MRE element interacts with other proteins as E1B-AP5 and poly(A)-binding protein C1 (PABPC1) (Massimelli et al., 2011). Coherent with these findings, PAN RNA was also found to interact in the nucleus with PABPC1. PABPC1 is a protein located in the cytoplasm of the infected host cells and responsible for modulating mRNA translation, maintaining their stability and processing. During

lytic phase of KSHV infection, SOX protein induces PAN RNA expression and PABPC1 relocation to the nucleus which is required for the expression of late KSHV gene, such as vIL-6 and k8.1 (Borah et al., 2011). Later, Massimelli further investigated the interaction relating PAN RNA to PABPC1 and ORF57 and found that PABPC1 actually suppresses PAN RNA expression and that ORF57 targets PABPC1 to enhance PAN RNA accumulation (Massimelli et al., 2013).

In fact, PAN RNA promotes the transition of KSHV infection from latent to lytic state through its interaction with the latency-associated nuclear antigen (LANA). LANA is important to maintain viral latency through inhibiting the expression of lytic genes. During latency, LANA is associated with a large portion of the KSHV episomes, while during reactivation, high levels of PAN RNA are transcribed to block LANAs interaction sites and induce its disassociation of LANA from KSHV episome, thus promoting viral gene expression (Campbell et al., 2014). The mechanism underlying PAN RNA regulation of viral gene expression appears to involve epigenetic pathways as well. PAN can bind promoters of viral genes and activate their expression such as the lytic regulator Rta, the demethylases JMJD3 (Jumonji domain containing 3) and UTX (ubiquitously transcribed tetratricopeptide repeat, X chromosome) and histone methyltransferase MLL2 (mixed-lineage leukemia protein 2) to induce the removal of the H3K27me3 mark and mark it to be activated. PAN RNA can also act as a repressor by interacting with SUZ12 (suppressor of zeste 12) and EZH2 (enhancer of zeste 2) which are components of the repressive PRC2 complex (polycomb repressive complex 2) and mediate the trimethylation of H3K27. Consequently, the elimination of repressive marks and activation of chromatin marks.

In addition to PAN RNAs, other lncRNAs have been reported during KSHV infection such as T3.0, T1.2, T1.5, T6.1, and ALT. ALT is identified in a recent study as a bona fide of KSHV with roles in viral gene expression. However, more studies are still needed to characterize lncRNAs that are reported in large-scale studies, to identify their exact molecular functions.

LONG NONCODING RNAS AND EPSTEIN–BARR VIRUS

EBV is a ubiquitous virus that is usually asymptomatic but causes malignant diseases in some cases such as nasopharyngeal carcinoma and lymphoma. Study on EBV transcriptome conducted by O'Grady et al. (2013) revealed that almost all the EBV genome is bidirectionally transcribed into several ncRNAs during the lytic phase.

As described by Marquitz et al., the BamHI A rightward transcripts (BARTs) are alternatively spliced and give rise to large wide of viral polyadenylated RNAs in Epstein–Barr virus–related epithelial cancers. The BART introns are the origin of 44 miRNAs while the exons are spliced and polyadenylated to form nonprotein-coding RNAs with nuclear localization that regulate infection in a similar mechanism of lncRNAs (Marquitz et al., 2015). In addition, two other nuclear lncRNAs, oriPtLs and oriPtRs, are transcribed during EBV reactivation and contribute to the viral gene expression and evasion of the host immune response (Cao et al., 2015). LOC553103 is another lncRNA that is associated with EBV-related cancer as it is responsible for promoting cancer cell migration and invasion. This lncRNA is regulated by a miRNA EBV-miR-BART6-3p (He et al., 2016). In a study on lymphoblastoid cell line, lncRNA profiling showed different patterns of expression of H19, H19 antisense, 7SL, and p53 mRNA. These are also released in the exosomes. Huang et al. used RNA-Seq and identified five lncRNAs with specific expression patterns to EBV-associated gastric cancer. Among these, SNHG8 showed interactions with several EBV proteins such as LF3, BHLF1, BHRF1, and BNLF2a and interacted with genes and pathways targeted by EBV such as transcription and mRNA metabolism (Huang et al., 2016).

OTHER EXAMPLES

Studies on other viruses also showed specific expression profiles of different lncRNAs following emerging viral infections. For example, in enterovirus 71 (EV71) infection, Yin et al. identified 160 enhancer-like lncRNA and mRNA pairs that are differentially expressed and hypothesized the enhancing role of these molecules upon neighboring genes. In addition, they used functional enrichment analysis and demonstrated the role of lncRNA–mRNA partners in many processes such as alternative splicing and acetylation (Yin et al., 2013). RNA-Seq analysis further confirmed by qPCR identified lncRNA BISPR and mRNA BST pair in hepatitis E virus (HEV) infection, as differentially expressed after 24 and 72 hour postinfection with HEV and correlated its upregulation with HEV viral replication.

Altogether, these findings highlight the different roles of lncRNAs in regulating the viral life cycle epigenetically, especially through regulation of viral gene expression, replication, and latency, and, thus, increasing viral pathogenesis. This is mediated by the interference of lncRNAs with viral and host genes.

LONG NONCODING RNAS IN VIRAL–HOST INTERACTION

The mechanisms of host immune systems are in constant evolution along with viral mechanisms of infecting different cellular organisms. Understanding both strategies and their interactions is of great importance in order to develop effective therapeutic approaches against viral infectious diseases with minimal inflammatory damage.

IMMUNE RESPONSE EMPLOYS LONG NONCODING RNAS AGAINST VIRUSES

Despite the extensive studies on host antiviral strategies, the exact underlying mechanisms are not fully understood. Studies on the involvement of lncRNAs in viral–host interactions added an additional layer of complexity and provided new insights into understanding responsible molecular pathways (Peng et al., 2010; Imamura et al., 2014; Li et al., 2017). A summary of main mechanisms is provided in Table 6.2.

First, Peng et al. (2010) correlated the expression levels of lncRNAs to the host antiviral immune response and suggested that lncRNAs may regulate immunity through their association with chromatin modifying complexes and the modulation of neighboring genes expression. Further studies were conducted to better understand this regulation. For example, Ouyang et al. showed that lncRNA NRAV negatively regulates INF-stimulated genes (ISGs) through a mechanism that appears to involve histone modification. During IAV infection, infected host downregulates lncRNA NRAV to allow ISGs production and repress viral replication (Ouyang et al., 2014). In addition, Barriocanal et al. reported that two lncRNAs, lncISG15, and lncBST2/BISPR, were induced by INF production during IAV and hepatitis C virus (HCV) infections, along with other ISGs such as BST2, IRF1, and ISG15. They found that lncBST2 positively regulates BST2/Tetherin, which have influence on INF release. On the other hand, Kambara et al. (2014) found that lncRNA-CMPK2 was actively upregulated after INF treatment and negatively regulates transcription of ISGs and they proposed a mechanism of this repression that involves RNA–protein interactions with chromatin modifying complexes or transcription factors. The negative regulation of IFN is important to maintain their controlled production and prevent inflammatory damage to the host (Fig. 6.1).

In addition, NEAT1, as mentioned previously, induces paraspeckle bodies' formation. SFPQ, a component of paraspeckles, binds to IL-8

gene promoter and represses its transcription. [Imamura et al. \(2014\)](#) found that NEAT1 activates IL-8 expression through a mechanism involving paraspeckles, as this lncRNA interacts with SFPQ and relocates it back to the paraspeckle, away from IL-8 promoter, which activates the expression of this cytokine. Recent findings indicate that NEAT1 employs the same mechanism and remove SFPQ from gene promoter to activate RIG-I expression and thus producing INFs ([Ma et al., 2017a,b](#)). [Morchikh et al.](#) further studied the role of NEAT1 in the regulation of immune system. In fact, they indicated NEAT1, in complex with HEXIM1, which is required for the assembly of HDP-RNP subunits composed of HEXIM1, DNA-PK, and paraspeckles. Subsequently, the HDP-PNP complex interacts with cGAS and PQBP1 to mediate immune response to pathogens ([Morchikh et al., 2017](#)).

[Atianand et al.](#) demonstrated the repression effect of long intergenic noncoding RNA (lincRNA)-EPS on immunity-related GTPase. They suggest different possible mechanisms such as blocking transcription via RNA-RNA interactions, direct interaction with target DNA sequences or by targeting genes through a ribonucleoprotein complex with hnRNPL ([Atianand et al., 2016](#)). Likewise, [Nishitsuji et al.](#) indicated that hnRNPU binds and stabilizes lncRNA#32. This lncRNA further binds to activating transcription factor to induce ISGs transcription, and, thus, repressing viral replication ([Nishitsuji et al., 2016](#)).

Using microarray analysis on mouse brains, [Li et al. \(2017\)](#) identified 1007 mRNAs and 518 lncRNAs during infection with Japanese encephalitis virus. Furthermore, Gene Ontology and network analysis revealed that the pathways associated with lncRNAs and mRNAs upregulation were related to antiviral innate immunity, inflammatory response, apoptotic, acetylation, and nucleotide binding. Moreover, the coexpression network analysis of selected lncRNAs and mRNAs indicated their interaction with three mRNAs, Nod1, Tap2, and Col4a1, which are involved in activating JNK cascade and NF κ B production ([Li et al., 2017](#)).

Collectively, these data show the important role of lncRNAs in mediating antiviral response of infected hosts.

LONG NONCODING RNAS MODULATE VIRAL EVASION OF IMMUNITY

In counterpart, viruses are continuously adapting their mechanism to evade antiviral immunity. Therefore viral pathogens hijack molecules in order to complete their life cycle and assure their survival. For example, [Xiong et al. \(2015\)](#) indicated that a lncRNA Lethe is activated by the transcriptional activator STAT3 in HCV-infected cells, represses IFN-1 produced by the antiviral immunity in order to promote HCV

replication. Moreover, HCV appears to activate proviral lncRNAs within their host. HCV induces the upregulation of lncRNA EGOT, also known as CSR32. Depletion of EGOT reduced viral replication by producing ISGs. In fact, many CSRs are upregulated upon HCV infection and development of liver cancer, which demonstrate that HCV employs cellular lncRNA EGOT to repress immunity and promote cell proliferation (Carnero et al., 2016).

Interestingly, viral pathogens encode their own lncRNAs as well. Thus knowing that viral genome is limited in length, viral encoded non-coding transcripts must play important roles for maintaining their life cycle and producing new progeny. Moreover, RNAs, especially lncRNAs, are more able to evade host immunity compared to protein elements. Therefore viruses might preferably employ these noncoding molecules to invade their hosts (Ding et al., 2016). Consistent with this hypothesis, Moon et al. found that flaviviruses encoded a lncRNA, referred to as sflRNA. This viral encoded lncRNA represses antiviral immune response through its interaction with RNA interference machinery, used by the host to degrade viral RNAs. Using coimmunoprecipitation analysis, they found an interaction of sflRNA with the RNase Dicer and Ago2, which are important players of RNA interference.

LONG NONCODING RNAS AS NEW CANDIDATES FOR VIRAL BIOMARKER AND THERAPY

As stated above, transcriptome analysis indicated that different expression patterns of large lncRNAs sets correlate to viral infection compared to normal controls. This raises the possibility of using such molecules as biomarker and to developing therapeutic strategies for viral diseases. In this chapter, we present related studies in the case of emerging viruses, represented mostly by HCV that has been mostly studied in recent years.

Several studies focused on lncRNAs related to HCV especially in HCC. Zhang et al. found significant difference in expression of seven lncRNAs between preneoplastic lesions and HCC. In comparison to dysplasia, LINC01419 and AK021443 lncRNAs presented elevated and decreased expressions, respectively, at early stage HCC. Further validation with qRT-PCR confirmed the significant overexpression of LINC01419 in HCC with hepatitis B virus (HBV) and HCV etiology compared to normal liver tissues (Zhang et al., 2015). Later, changes in expression levels of eight lncRNAs were significantly related to HCC compared to adjacent normal tissues. Notably, the aberrant expression of lncRNAs was specific to the related hepatitis virus, as lncRNA

TABLE 6.2 Mechanisms of Long Noncoding RNAs (lncRNAs) in Antiviral Immune Response

Immunity pathway	Related lncRNA(s)	Mechanism	Author/year
Interleukins	Nonidentified	<ul style="list-style-type: none"> • Chromatin modification • Regulation of neighboring genes 	Peng et al. (2010)
Activation of interleukins	NEAT1	Relocation of SFPQ to paraspeckles	Imamura et al. (2014)
Suppression of ISGs	NRAV	Histone modification	Ouyang et al. (2014)
Suppression of antiviral interference	sRNA	Interaction with Dicer and Ago2	
Activation of ISGs	lncRNA#32	ATF2	
Repression of IRGs	LincRNA-EPS	<ul style="list-style-type: none"> • Chromatin remodeling • Interaction with heterogeneous nuclear ribonucleoprotein L 	Atianand et al. (2016)
Activation of RIG-I	NEAT1	Relocation of SFPQ to paraspeckles	Ma et al. (2017a,b)
Inflammatory response	E52329 and N54010	Reducing JNK and MKK4 phosphorylation	Li et al. (2017)
DNA-mediated immunity (cGAS–STING–IRF3 pathway)	NEAT1	Formation of a complex with HEXIM1, DNA-PK, and paraspeckle	Morchikh et al. (2017)

ATF2, Activating transcription factor 2; *IRGs*, immunity-related GTPase; *ISGs*, interferon-stimulated genes; *LincRNA*, long intergenic noncoding RNA; *LncRNA*, Long noncoding RNA; *RIG-I*, retinoic acid-inducible gene I.

PCAT-29 was correlated to HBV, while aHIF and PAR5 were related to HCV and Y3 to hepatitis D virus–induced HCC ([Zhang et al., 2016](#)).

Interestingly, recent studies suggest potential use of lncRNAs as serum biomarkers for HCV-induced HCC. As reported by Kamel et al. in their study of lncRNAs expression in serum of 160 participants, expression patterns of lncRNA–UCA1 and lncRNA–WRAP53 were significantly different among sera of HCC, chronic HCV infection, and healthy controls, with a higher expression in advanced stage of HCC. They further found improved sensitivity when combining the studied lncRNAs expression with serum alpha-fetoprotein level ([Kamel et al., 2016](#)). Rheumatoid arthritis (RA) is also induced by HCV. Using qRT-PCR, elevated levels of lncRNA-AF085935 in sera of HCV-induced RA

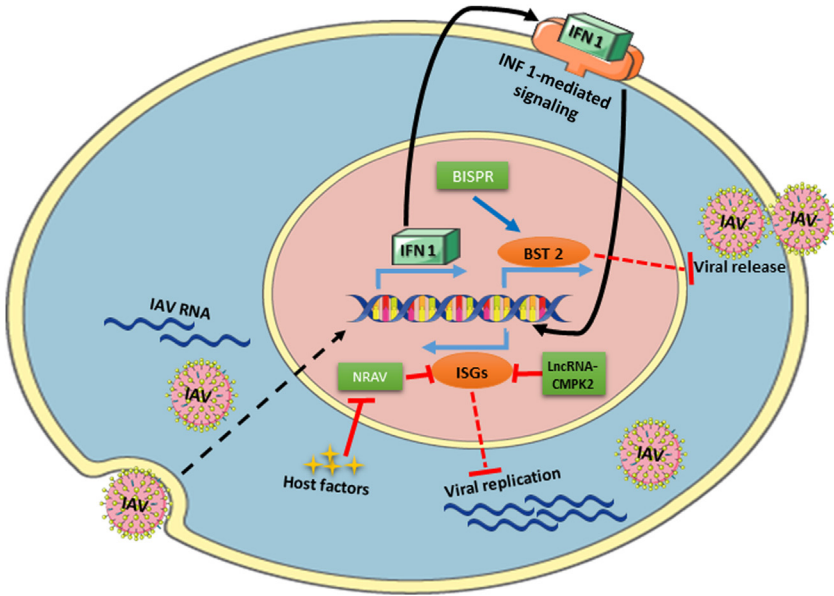


FIGURE 6.1 Following IAV infection, the infected cell downregulates NRAV, which is a negative regulator of ISGs, in order to allow ISGs activation and thus repressing viral replication. On the other hand, lncRNA-CMPK2 represses ISGs, which is important to maintain the balance of inflammatory response and prevent damage to the cell. IAV, Influenza A virus; ISGs, interferon-stimulated genes; lncRNAs, long noncoding RNAs.

patients were reported, suggesting lncRNA-AF085935 as candidate biomarker for early detection of HCV-related RA (Sabry et al., 2017). Another lncRNA, referred to as ncRNA-HEIH, is expressed in a different manner in sera and exosomes during HCV infection and is proposed as an indicator of HCC. In fact, reported expression levels of this lncRNA were higher in HCV-induced HCC patients compared to those in HCV-induced cirrhosis patients and less expression levels were observed in chronic hepatitis C patients (Zhang et al., 2017).

Recently, a study using microarray analysis to detect expression changes of coding and noncoding transcripts in HIV-1 and HIV-2-infected monocyte-derived macrophages revealed significant different expression profiles. Actually, chr2: 165509129-165519404 and lincRNA: chr12: 57761837-57762303 correlated better to HIV-1 infection and lincRNA: chr10:128586385-128592960, XLOC_001148 and lincRNA: chr5:87580664-87583451, related more to HIV-2 infection.

These data reveal significant importance of lncRNAs as new biomarker for detection, diagnosis, and prognostic of viral pathologies. However, these efforts have to extend to other viral pathologies, and more investigations are needed to further confirm their specificity and sensitivity.

CONCLUSION

Existing data on the lncRNAs associated to viral infections give more insight to cellular pathways involved in their regulation. In addition, large-scale transcriptomic studies identified thousands of lncRNAs that need to be studied and characterized to better understand their mechanism of action. This is providing a novel and promising research field that can lead to new molecular network and pathway discoveries, enabling a deeper understanding of these pathologies. Moreover, the tissue specificity and the difference of expression patterns of lncRNAs among different viral infections suggest promising roles as diagnosis biomarkers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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