Noncoding RNA Expression During Viral Infection: The Long and the Short of It

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Abstract New technologies have expanded our view of viral–host interactions with the growing identification of non-coding RNAs (ncRNAs) that act as key regulators of viral infection. In this chapter, we explore novel genomics-based approaches used to characterize ncRNAs involved in viral infection, focusing mainly on microRNAs and long noncoding RNAs. We present recent evidence implicating virally encoded and host-derived ncRNAs in viral replication and pathogenesis regulation, focusing on four different viral diseases (IAV, KHSV, HIV, and HBV). Finally, we discuss the current knowledge of ncRNAs modulation of innate and adaptive immune responses to viral infection. These findings highlight the complexity of host–pathogen networks determining the outcome of viral disease. Understanding the role of ncRNAs in these networks may offer novel antiviral therapy and diagnostic tools.

1 Introduction

Less than 2 % of the mammalian genome encodes protein-coding genes, while the majority of the genome is pervasively transcribed. This suggests that most of the mammalian transcriptome consists of noncoding transcripts. Noncoding RNAs (ncRNAs) comprised of microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and Piwi-interacting RNA (piRNA), to name a few, regulate a variety of diverse cellular and organismal processes. Since the discovery of RNA interference in nematodes 20 years ago [1–3], ncRNAs have garnered a great deal of attention, in

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particular for their role in pathogen–host interactions. To date, there are over 24,000 miRNA loci from 206 species registered with miRBase, a primary miRNA sequence repository that has grown tremendously with small RNA deep sequencing [4]. LncRNAs have been emerging more recently as key regulators of diverse cellular processes, including viral infection. In general, they are defined as transcripts that have at least 200 nucleotides and which lack any positive-strand open-reading frames longer than 30 amino acids. The number of lncRNAs is still a matter of debate, with, in humans, between 9,277 manually annotated lncRNA genes producing 14,880 transcripts present in GENCODE [5], to 56,018 lncRNA genes producing 95,135 transcripts in NONCODEv4 [6]. The nomenclature of lncRNAs is constantly evolving and they have been classified according to their location with respect to protein-coding genes: intergenic (lincRNA), antisense, sense exonic, and sense nonexonic [6]. In contrast to miRNAs that have a well-defined function in the cytoplasmic compartment by translational inhibition and/or degradation of target mRNAs, lncRNAs have a wide range of functions through diverse molecular mechanisms (reviewed in [7]). This chapter aims to summarize our current knowledge of miRNA and lncRNA roles in the context of viral infection in mammals, with a specific focus on ncRNA regulation of viral replication and immune responses.

2 Virus-Encoded ncRNAs

Mammalian viruses are highly diverse etiologic agents that can cause an array of human disease. Virus replication and the cellular responses activated to combat infection coordinately determine virus-induced pathogenesis. Similar to what is found in the host genome, viruses can encode small noncoding transcripts, or virally encoded miRNAs, that are more prevalent in DNA viruses due to their much larger genomes and their ability to replicate in the nucleus and cause persistent infection.

Virus miRNA expression was first reported in the context of Epstein–Barr virus (EBV) infection. Small RNAs cloned from a Burkitt's lymphoma cell line latently infected with EBV were found to originate from the virus including two miRNAs from the introns of the BARTS (BAMHI A rightward transcripts) [8]. Since then, more than 200 viral miRNAs have been identified from large DNA viruses of the *Herpesviridae* family. Similar to host-derived miRNAs, virally encoded miRNAs are approximately 22 nucleotides in length and primarily regulate gene expression by binding to sequences located in the $3'$ untranslated region (UTR) of target mRNAs through their "seed" region. Viral lncRNAs, on the other hand, are often smaller than 200 nucleotides that have been described for host lncRNAs. There are several novel classes of viral ncRNAs, including influenza A virus small viral RNAs (svRNA) [9], West Nile virus subgenomic flavivirus RNA (sfRNA) [10], Herpesvirus saimiri small nuclear RNAs [11], and the chimeric HBx-LINE1 viral-human gene fusion transcript, that functions as a hybrid RNA [12]. Retroviral antisense ncRNAs, such as HIV antisense lncRNA, regulate viral transcription by directing epigenetic silencing complexes to the LTR [13]. The HBZ antisense transcript of human T-lymphotrophic

virus 1 (HTLV-1) is consistently expressed in adult T-cell leukemia/lymphoma (ATL) cells and codes for a multifunctional protein that positively correlates with proviral load and disease severity associated with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [14]. The roles of these ncRNAs in viral replication and pathogenesis remain poorly understood; however, HBZ antisense transcript, for example, could be a predictive biomarker of disease.

Viral ncRNAs are capable of regulating a variety of viral and cellular processes, including viral latency, apoptosis, and immunity. For instance, Kaposi's sarcoma-associated herpesvirus (KSHV) encodes over 90 genes and has an abundant number of miRNAs, 12 of which are expressed from two latency-specific promoters that regulate the expression of latent viral proteins ORF71, ORF72, ORF73, and KapB controlling KSHV latency [15]. KSHV-encoded miRNAs can regulate cellular transformation and tumorigenesis [16] and target TLR-responsive genes, *IRAK1* and *MYD88*, to reduce herpesvirus-induced inflammation [17]. Virally encoded miRNA mimics of cellular miRNAs have also been discovered. For example, KSHV miR-K12-11 is highly homologous to cellular miRNA-155, known to function as an oncogene, with both miRNAs containing identical seed regions critical for target recognition and downregulating a common set of mRNA targets [18]. The viral miR-155 analog is thought to contribute toward KSHV-associated malignancies in infected patients, a viral mimic strategy similar to herpes simplex virus 1 (HSV-1) $\gamma_1 34.5$ protein with homology to cellular GADD34 controlling host protein synthesis [19]. In addition to human gammaherpesviruses, DNA virus families with members known to encode miRNAs include *Polyomaviridae*, *Adenoviridae*, *Ascoviridae*, and *Baculoviridae* (reviewed in [20]). The current knowledge of virally encoded ncRNAs and their cellular targets is summarized in Table 1.

3   Genomics-Based Approach to Identify and Characterize Host-Encoded ncRNAs Involved in Host-Response to Viral Infection

Initial studies of ncRNAs were based on conventional molecular biology techniques including RT-PCR and northern blot, characterizing the expression of single ncRNAs. The landscape of ncRNAs has widely expanded with recent advancements in sequencing technologies and computational biology.

3.1   Methods for Genome-Wide Characterization of ncRNA Expression

Large scale gene expression analysis has been dominated by DNA microarray since its development in the 1990s. Array technologies provide simultaneous

Table 1 Viral-encoded ncRNAs and their cellular target genes and pathways

Virus family or subfamily	Virus	ncRNA type	ncRNA name	Functional role and cellular targets	Reference (s)
Retroviridae	HIV	lncRNA	Antisense transcript	Epigenetic regulation of gene expression	[13]
	MLV		U3 antisense transcripts	Insertional activation of cellular proto-onco-genes *Jdp2* and *Bach2*	[21]
	HTLV-1		*HBZ* transcript	Promotes T-cell lymphoma; CREB-mediated inhibition of cyclin D1; regulates hTERT transcriptional activity	[22–24]
Gammaherpesviridae	EBV	miRNA	ebv-miR-BART6-5p	DICER	[25]
			ebv-miR-BART5	Modulator of apoptosis PUMA	[26]
			ebv-miR-BART5	Pro-apoptotic protein BIM	[27]
			miR-BHRF1	Promotes cell cycle progression and prolifera-tion; inhibits apoptosis	[28]
			miR-BHRF1-3	CXCL-11	[29]
		lncRNA	EBER-1 (166 nt) and EBER-2 (172 nt)	Promotes cellular proliferation and transforma-tion; inhibits apoptosis	[30]
	KHSV	miRNA	miR-K12-10a	TWEAKER	[31]
			miR-K12-1, 3 and 4-3p	Targets CASP3 to regualte apoptosis	[32]
			miR-K12-9-5p and miRK12-7-5p	KSHV RTA	[33, 34]
			miR-K12-11	targets IKKε to modulate IFN signaling	[35]
			miR-K12-1	IkB-alpha	[36]
			miR-K12-1, miR-K12-6-5p, miR-K12-11	MAF	[37]
			miR-K12-4-5p	Targets Rbl2 to regualte global epigenetic reprogramming	[38]
			miR-K12-9 (miR-K9) and miR-K5	IRAK1 and MYD88	[17]
			miR-K1, miR-K3-3p, miR-K6-3p, miR-K11	THBS1	[39]

Family	Virus	RNA type	ncRNA	Target / Function	Reference
			miR-K1	p21	[40]
			miR-K12-3, miR-K12-7	C/EBPbeta p20 (LIP)	[41]
		lncRNA	miR-K12-11	BACH1, FOS	[18, 42]
			lncRNA-PAN	Multifunctional transcript regulating viral replication and host response	[43, 44]
	MCMV	miRNA	miR-M23-2	CXCL16	[45]
	HCMV	miRNA	miR-US25-1	CCNE2, H3F3B, TRIM28	[46]
	HCMV, KSHV, EBV	miRNA	miR-UL112, miR-K12-7, miR-BART2-5p	MICB	[47, 48]
			miR-K5; miR-K9; miR-K10a/b	BCLAF1	[49–51]
	HVS	snRNAs	*H. saimiri* U-rich nuclear RNAs (HSURs)	HSUR 1 and 2 bind specific host miRNAs in virally transformed T cells. HSUR 1 downregulates cellualr miR-27a	[11]
Alphaherpesviridae	MDV1	miRNA	miR-M4	PU.1	[52]
			miR-M4	PU.1, GPM6B, RREB1, c-Myb, MAP3K7l, P2 PU.1, C/EBP	[53]
			miR-M3	SMAD2	[54]
Flaviviridae	WNV	lncRNA	Noncoding subgenomic flavivirus RNA (sfRNA)	Required for viral pathogenicity and evasion of the type I interferon response	[10]
Orthomyxoviridae	IAV	miRNA	Influenza A virus-derived small viral RNAs (svRNAs)	Interacts with the viral RdRNP to initiate the switch from viral transcription to replication	[9]

quantification of tens of thousands of transcripts and have been widely used to characterize transcriptomic responses of cells, animal models, and human patients to virus infection (reviewed in [55]). Accurate detection and quantification of miRNAs poses several challenges that include the lack of common sequences for their purification, the high sequence similarity among miRNA within the same family, and the presence of natural variants of miRNAs, called isomiRs, which result from post-transcriptional nucleotide additions or deletions to 3′ and 5′ ends of mature miRNAs (reviewed in [56]). Various microarray-based strategies for profiling miRNA expression have been developed, including different approaches for fluorescent labeling of the miRNA for subsequent hybridization to classical DNA-based probes or locked nucleic acid (LNA)-modified capture probes (reviewed in [56]). LncRNA-specific microarrays offer less technical variations and they mainly differ by the number of lncRNAs targeted by DNA probes present on the chip. At the time of writing, lncRNA microarrays include Agilent® custom arrays by GENECODE targeting 11,880 human lncRNA transcripts [5] (22,001 lncRNAs transcripts in the newest version), Arraystar® LncRNA human and mouse arrays targeting ~30,600 and ~31,423 lncRNA transcripts, respectively, and Affymetrix® Human Gene ST Array covering 11,000 lncRNA RefSeq transcripts. The main limitations of microarray-based characterization of ncRNA are a restricted linear range of quantification and their dependence on the prior knowledge of annotated transcripts for probe design. In addition, microarrays can have imperfect specificity in some cases for miRNAs that are closely related in sequence.

The recent development of next-generation sequencing (NGS) platforms has enabled a novel approach for ncRNA expression profiling by RNA-Seq. RNA-Seq relies on the preparation of a cDNA library from the RNA sample of interest, followed by the "massively parallel" sequencing of millions of individual cDNA molecules from the library. The method of cDNA library construction used determines the type of transcripts to be sequenced: small RNA-Seq for sequencing of miRNAs, small nuclear (sn), small nucleolar (sno), and piwi-associated (pi)RNAs; mRNA-Seq for sequencing various types of polyadenylated transcripts; and total RNA-Seq for sequencing whole transcriptomes (all transcripts after ribosomal RNA (rRNA) removal). Remarkably, as many lncRNA transcripts may not be polyadenylated [57, 58], it is important to use total RNA-Seq approaches for the comprehensive detection of lncRNAs. Compared to microarray, RNA-Seq has a wider dynamic range, higher precision, and reproducibility [59–61], and is able to distinguish ncRNA that differ by as few as one nucleotide. Importantly, because RNA-Seq does not require prior transcript annotation for probes, novel transcripts can be detected, including both protein-coding and ncRNA transcripts, as well as RNA with somatic mutations and alternative splicing forms [62, 63]. The main limitations of RNA-Seq are the computational infrastructure required for data analysis, interpretation, and storage. The cost associated with sequencing can also be a limiting factor, though newer technologies, such as Illumnina NextSeq, are enabling quicker run times to generate up to 400 million clusters with a more cost-effective system that is used to store, analyze, and share genomic data.

3.2   Methods to Computationally Infer ncRNA Function

3.2.1   miRNA Annotation, Analysis, and Target Prediction

A number of tools are available for miRNA annotation, analysis, and target prediction (reviewed in [64]). The miRBase database is the primary online repository for miRNA sequences and annotation [4]. Several software packages predict novel miRNAs from RNA-Seq data, including miRDeep [65] and mirTools [66]. Finally, many miRNA target prediction tools have been published. These tools indirectly predict miRNA function, as miRNAs binding to the 3′ UTR of their mRNA target lead to the degradation or the translational repression of the target mRNA. Target prediction tools are based on sequence comparisons between mature miRNA and 3′ UTRs in candidate mRNA targets. Three widely used algorithms that have enhanced mRNA target prediction include TargetScan [67], miRanda [68], and DIANA-microT [69]. The best algorithms currently identify 60 % of all available targets and provide one valid target in approximately every three predicted targets [64], highlighting the importance of experimental validation.

3.2.2   lncRNA Annotation and Function Prediction

Many online databases are available for lncRNA annotation, some of which include predicted or experimentally validated lncRNA functions [6, 70–75]. Identifying lncRNA functions is especially challenging because of the large number of lncRNAs and the variety of mechanisms driving their functions. With the large amount of transcriptome data created by microarray and RNA-Seq technologies, predicting gene function on the basis of expression has been proposed as an attractive strategy [76]. One approach for predicting function of both coding and noncoding genes is the "guilt by association" approach, which relies on finding which RNAs have similar expression profiles to protein-coding genes of known function. Several methods have been used to infer co-expression networks with the objective of predicting gene function: algorithms using the concept of mutual information (MI) [77, 78]; random matrix theory (RMT) [79]; and correlation analysis such as weighted gene co-expression network analysis (WGCNA) [80, 81]. This strategy was applied to mouse lncRNAs after re-annotation of the Affymetrix Mouse Array using 34 datasets derived from diverse mouse tissues and allowed the functional annotation of 340 lncRNAs mainly involved in development, cellular transport, and metabolic processes [81]. A similar method was also applied for lncRNA function annotation based on RNA-Seq data [82]. Many intergenic long noncoding RNAs (lincRNAs) that were co-expressed with genes in the p53 pathway were further validated as p53 transcriptional targets, including lincRNA-*p21* which serves as a repressor in p53-dependent transcriptional responses [83]. Similarly, Hu et al. inferred potential functions of lincRNAs in T cells by analyzing their co-expression with coding genes and showed that the expression of LincR-*Ccr2*-5′AS was correlated with the expression of genes within a chemokine-mediated
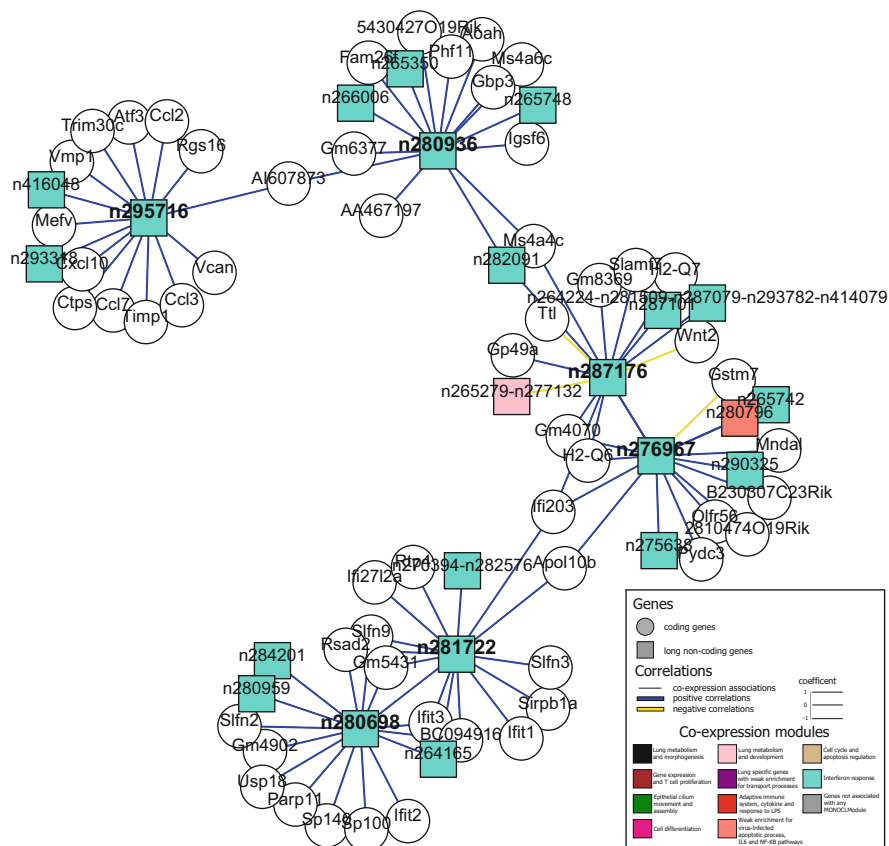
Fig. 1 Example of six lncRNAs induced in mouse lung after respiratory virus infection and co-expressed with interferon response genes. These lncRNAs: n280936, n280698, n281722, n276967, n295716, and n287176, depicted in bold in the figure, also have binding motifs for IRF9 in their promoters. The network shows the co-expression associations between each lncRNA and top 15 most correlated genes. Nodes in the graph represent the coding or lncRNA genes while edges represent the significant co-expression associations. Based on whole network topology, n280936, n280698, and n295716 were classified as hubs of the interferon response module. The lncRNA-coding genes co-expression network was visualized using the Mouse NOnCode Lung database (MONOCLdb) at http://www.monocldb.org/. The MONOCLdb contains annotations, expression profiles, and functional enrichment results of lncRNA expressed in Collaborative Cross founder mice in response to respiratory infection caused by influenza and SARS-CoV [52]

signaling pathway [84]. The knockdown of LincR-*Ccr2-5′*AS decreased the expression of its neighboring chemokine receptor-encoding genes.

Recently, we predicted the function of 5,329 lncRNAs involved in pulmonary responses to influenza A virus or SARS-CoV infection by using WGCNA and two levels of annotation: (1) a coarse annotation based on lncRNA membership within a co-expression module and (2) a finer rank-based annotation method [85]. This analysis identified many lncRNAs induced after viral infection and that were closely associated with interferon (IFN) response genes (Fig. 1).

3.3   Genome-Wide Characterization of ncRNA Expression During Viral Infection

Microarrays have been widely used to characterize miRNA expression in response to viral infections, with more than 100 publications referenced in PubMed to date. Among them, analysis of miRNAs expressed in the lung of mice following infection with influenza A virus found 130 cellular miRNAs were differentially regulated, with distinct expression patterns in response to highly pathogenic 1918 H1N1 virus compared to a nonlethal seasonal H1N1 influenza virus [86]. Some of these miRNAs had predicted mRNAs targets with anti-correlated expression levels, such as miR-200a and *sip1*, that were enriched in immune response and cell death pathways, suggesting that type I IFN singaling and CREB activity linked with the high virulence of 1918 pandemic influenza virus may be regulated by miRNAs.

RNA-Seq analysis has also been used to study the ncRNA response to respiratory virus infection. Peng et al. identified over 1,500 lncRNAs and 200 small RNAs, such as snRNAs and piRNA, expressed in mouse lung in response to SARS-CoV or influenza A virus [87, 88]. Using a large RNA-Seq dataset consisting of a wide-range of pulmonary transcriptional responses during SARS-CoV and influenza infection, we have expanded upon this analysis and identified 5,329 lncRNAs differentially expressed after infection in the lungs of 8 genetically diverse mouse strains [85]. These lncRNAs accounted for about 40 % of total genes differentially expressed upon viral infection. Most of the upregulated lncRNAs were related to the innate immune response and were co-expressed with genes specific to immune cells, suggesting they might be associated with immune cell infiltration of the lung following infection (Fig. 1). Some of these lncRNAs were highly connected (hubs) in the interferon-response co-expression module and could therefore have a role in controlling type I IFN signaling during viral infection [85].

Finally, RNA-Seq analysis of ncRNA expression in a CD4+ T cell line identified over 1,000 lncRNAs and 531 miRNAs differentially expressed upon HIV-1 infection [88, 89]. Integration of mRNA-Seq and small RNA-Seq data identified 5,023 anticorrelated interactions involving 46 differentially expressed miRNAs predicted to target as many as 518 mRNAs, including target genes with transcription regulatory activity, such as P/CAF (P300/CBP-associated factor), and genes within T cell activation-related pathways. The downregulation of miRNAs may have contributed to increased T cell receptor signaling.

4   Role of Host and Viral ncRNA in Regulating Viral Replication and Pathogenesis

Host and viral ncRNAs can impact viral replication and pathogenesis. For example, ncRNAs can influence poliovirus tissue tropism [90], Coxsackie A21 virus-induced myositis [91], hepatotoxicity associated with oncolytic adenoviruses [92], and HIV control of HLA-C expression [93]. Here, we focus on how host and viral ncRNAs

impact the replication or pathogenesis of influenza A virus, KSHV, HIV, and HBV infection associated with hepatocellular carcinoma.

4.1 Influenza A Virus

Influenza virus is a respiratory pathogen that causes significant morbidity and mortality, with over 200,000 people hospitalized from infection each year in the United States alone. Influenza H1N1 and H3N2 viruses circulate within the human population and on occasion new viruses emerge as a result of gene reassortment, the shuffling of viral gene segments from different influenza A viruses. This in turn can give rise to a worldwide pandemic, as demonstrated by the recent 2009 pandemic H1N1 influenza virus that originated in swine due to a reassortment event between swine H1N1 and human H3N2 viruses.

4.1.1 Influenza svRNA

It has been generally accepted that RNA viruses do not transcribe ncRNAs; however, this notion is beginning to change with NGS technologies. For influenza A virus (IAV), a segmented negative-strand RNA virus, deep sequencing has captured small viral RNAs (svRNA) varying between 22 and 27 nucleotides in length originating from all eight genomic segments of the virus. The production of svRNAs is dependent on the IAV RNA-dependent RNA polymerase (RdRp), comprised of polymerase subunits, PB1, PB2, and PA, and corresponds to the shift from IAV transcription to replication. Unlike other virally encoded small ncRNAs, IAV svRNAs do not induce host antiviral defenses (e.g., IRF3 activation or IFNβ induction), but they were found to be important for viral replication. For instance, the use of LNA complementary to segment 4 svRNA (HA svRNA) significantly reduced HA vRNA synthesis resulting in decreased HA protein levels and reduced influenza H1N1 virus replication in cell culture [9].

4.1.2 Host lncRNA Regulation of Influenza Replication

VIN is a nuclear lncRNA, the expression of which is strongly increased 10–60-fold in human lung epithelial cells in response to H1N1, H3N2, or H7N7 IAV infection [94]. Interestingly, this lncRNA is not increased following infection with influenza B virus, IFNβ treatment, or TLR3 stimulation by polyI:C. Silencing of *VIN* decreases influenza virus replication suggesting that VIN could be a proviral factor, but its mechanism of action has not yet been determined. Silencing of another lncRNA named *NRAV* was found to decrease influenza virus replication in vivo and in vitro. However, *NRAV* was downregulated following viral infection, which increasing ISG induction and thereby inhibiting influenza virus replication [95].

4.2   Human Immunodeficiency Virus

There are more than 35 million people living with HIV in the world today. The retrovirus targets CD4+ T cells and their depletion facilitates a patient's progression toward acquired immunodeficiency syndrome (AIDS) if left untreated. Mucosal transmission is one of the primary routes of HIV transmission and mucosal immune responses have been suggested to control systemic infection after virus exposure. Deep sequencing is revealing new insight into mucosal immunity against HIV and in particular, changes in ncRNA expression and their potential role in viral pathogenesis and HIV-associated disease.

4.2.1   Host ncRNAs Involved in Mucosal Immunity Against HIV

MiRNAs regulate intestinal epithelial differentiation, architecture, and barrier function [96], which are disrupted during HIV infection in part due to the rapid depletion of CD4+ T cells in the gastrointestinal tract [97]. In a nonhuman primate model of AIDS, simian immunodeficiency virus (SIV) infection decreased the expression of mucosal miRNAs (e.g., miR-16, -194, and -200c) involved in epithelial homeostasis of the gut and coincided with increased 5′–3′-exoribonuclease 2 (XRN2) protein expression and altered levels of miRNA biogenesis machinery components, DICER1 and Argonaute 2 (AGO2) [98]. In this same study, miRNA profiled from total RNA extracted from jejunal biopsy specimens from HIV-infected and HIV-negative patients also found reduced intestinal miRNA expression in patients chronically infected with HIV. These findings suggest that the gut mucosal response to viral infection involves decreased miRNA expression likely impairing epithelial cell growth and development during SIV and HIV infections. In a separate study, co-expression analysis between differentially expressed lncRNAs and coding genes revealed the downregulation of transcripts in rectal mucosa of rhesus macaques infected with SIV that were associated with wound healing, cell–cell adhesion, and tissue formation during acute SIV infection [99]. Taken together, alteration of the local microenvironment in the small intestine through ncRNA downregulation could lead to the dissemination of virus and intestinal enteropathy.

4.2.2   Viral Antisense lncRNA Involved in HIV Latency

After entering a cell, retroviruses integrate into the host genome where viral genes can be transcribed or remain latent. In addition to viral genomic sense RNA and mRNA, several retroviruses, including HIV, encode antisense RNAs (asRNAs) that are transcribed during infection (Table 1). Kobayashi-Ishihara et al. report the transcription of an apparent major form of HIV asRNAs, *ASP-L*, in HIV-1-infected cells. This transcript is localized in the nucleus and inhibits HIV-1 replication

[100]. The molecular mechanism of HIV asRNA action was recently elucidated [13]. It is involved in the epigenetic regulation of viral transcription through its recruitment of DNMT3a and possibly other chromatin-remodeling proteins, such as enhancer of Zeste 2 (EZH2) and histone deacetylase 1 (HDAC-1), to the viral promoter (5′LTR). This complex induces a repressive chromatin state, which epigenetically silences the transcription of viral genes. Together with viral asRNAs, cellular lncRNAs may also be involved in regulating proviral HIV latency, as many lncRNAs function as epigenetic regulators [101, 102].

4.2.3   Host lncRNA Controlling HIV Replication

A host lncRNA named *NEAT1* was shown to directly control HIV replication. *NEAT1* is an essential structural component of paraspeckles, which are nuclear bodies rich in RNA-binding proteins and splicing factors. Recently, *NEAT1* was found upregulated in human cell lines infected with HIV-1 [103]. Silencing of *NEAT1* decreased nuclear paraspeckle bodies and was associated with enhanced HIV expression and increased levels of unspliced HIV transcripts in the cytoplasm. Zhang et al. hypothesized that *NEAT1* modulates HIV-I replication by promoting HIV mRNA nuclear sequestration in paraspeckles; however, Imamura et al. recently described that silencing of *NEAT1* impairs the induction of numerous genes including antiviral factors following TLR3 stimulation [104]. Therefore, the effect of *NEAT1* on HIV replication may also be indirect and mediated by dysregulation of the antiviral host response.

4.3   *Kaposi's Sarcoma-Associated Herpesvirus*

Kaposi's sarcoma-associated herpesvirus KHSV is a gammaherpesvirus causing several human cancers and lymphoproliferative disorders, including Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and a subset of multicentric Castleman's disease (reviewed in [105]). Like other herpesviruses, KSHV infection is characterized by two states: viral latency and lytic growth. During latency, very few viral genes are expressed, reducing the number of viral epitopes available to trigger a host immune response. KSHV latent genomes are bound to host histones and can form either minichromosomes or episomes, which are epigenetically regulated [106]. Upon stimulation by various stress responses, KSHV activates the lytic program of its replication cycle to produce new infectious viral particles. Disruption of the repressive viral chromatin state is essential for KSHV reactivation, and it is mediated by viral proteins and ncRNAs [106].

4.3.1 miRNA Regulation of KSHV Replication

Early and late miRNA expression in primary lymphatic endothelial cells (LECs) infected with KSHV regulates the antiviral response by facilitating viral gene expression. Early-response miR-132 is highly upregulated after infection, which affects viral gene expression by suppressing transcriptional co-activator *p300*. Silencing of *p300* in LECs impairs antiviral responses to KSHV by decreasing *IFNβ*, *ISG15*, *IL-1β*, and *IL-6* mRNA expression. Conversely, the inhibition of miR-132 induction suppresses KSHV replication in LECs, resulting in increased IFNβ mRNA levels. A similar phenomena is observed with herpes simplex virus-1 (HSV-1)- and human cytomegalovirus (HCMV)-infected monocytes where virus-induced host miR-132 inhibits *p300* expression [107].

4.3.2 lncRNA Control of KSHV Replication

KSHV encodes a viral lncRNA known as polyadenylated nuclear RNA (*PAN RNA*), which was discovered 18 years ago as the most abundant transcript produced during the lytic phase [108]. Several reports suggest that *PAN RNA* is a multifunctional regulatory transcript and plays an important role in KSHV replication [43, 44, 109–111]. *PAN RNA* interacts with several host factors, including histones H1 and H2A and chromatin modifiers, such as lysine demethylases UTX and JMJD3, and the histone methyltransferase MLL2 [109]. These interactions remove H3K27me3, a repressive chromatin marker within the KSHV genome [109]. *PAN RNA* also mediates changes in histone modifications by binding the Polycomb repressive complex 2 (PRC2) [110]. In addition, *PAN RNA* interacts with the viral latency-associated nuclear antigen (LANA), which is a transcriptional repressor associated with latent viral episomes. Upon reactivation, *PAN RNA* sequesters LANA away from the viral episome [44]. Finally, *PAN RNA* interacts with the host poly(A)-binding protein C1 PABPC1, which allows late viral mRNA exportation and translation. In addition to its role in regulating viral replication, *PAN RNA* also acts on cellular gene expression, modulating the expression of genes involved in cell cycle, immune response, and production of inflammatory cytokines [43, 110]. To date, there are no known host lncRNAs reported to directly regulate KSHV replication. However, similar to HIV, host lncRNAs that regulate chromatin state could also potentially control KSHV episomal latency [101, 102].

4.4 Hepatocarcinoma Associated with Hepatitis B Infection

Hepatitis B virus (HBV) is an oncogenic virus belonging to the *Hepadnaviridae* family of DNA viruses. HBV infection is the leading cause of acute and chronic hepatitis B, liver cirrhosis, and it is a major risk factor of hepatocellular carcinoma

(HCC). Nearly 2 billion people are infected with HBV worldwide and more than 350 million are reported to be chronic HBV carriers. Many host cellular ncRNAs are involved in HCC (reviewed in [112]) in addition to specific viral ncRNA that can facilitate oncogenesis.

4.4.1   Viral-Human Chimeric lncRNA

After infection, HBV not only replicates as an episome, but HBV DNA can also integrate into the host genome, leading to chromosomal rearrangements and deletions. In most integrated subviral DNAs, X-ORF is maintained and the HBx gene transcribed at low levels during acute and chronic hepatitis (V. Schluter et al., Oncogene 1994). Such integration has been linked to liver cancer formation, with 85–90 % of HBV-associated HCC tumors having at least one HBV insertion [113]. Recently, Lau et al. detected a specific integration site of HBV into a transposable *LINE1* element in chromosome 8 for about 23 % of HBV-HCC cases in a cohort of 90 patients [12]. [114] This integration leads to a chimeric viral-human transcript, HBx-LINE1. Silencing of *HBx-LINE1* inhibits cell motility and Wnt/β-catenin signaling. The HBx-LINE1 fusion transcript encodes for a fusion protein; but unexpectedly, its oncogenic effect is dependent on the *HBx-LINE1* mRNA and not protein expression. It was therefore concluded that *HBx-LINE1* functions as a lncRNA. Moreover, *HBx-LINE1* transgenic mice had increased risk of HCC development proving the oncogenic role of the *HBx-LINE1* transcript.

### 4.4.2   Host lncRNA Regulating HBV- and HCV-HCC

Several cellular lncRNAs are differentially expressed in HCCs, including *MALAT1*, *HULC*, *H19*, *HEIH*, *HOTAIR*, *MEG3*, *uc002mbe.2*, *lncRNA-LET*, *MVIH*, and *Dreh* (reviewed in [115]). Interestingly, these lncRNA could provide prognostic and diagnostic markers of HCC [112]. Most of these lncRNAs have general oncogene-like effects and only two of them, HULC and Dreh, have been directly linked to HBV infection. Highly Upregulated in Liver Cancer (*HULC*) is upregulated in HBV-HCC and correlated with HBx levels [116]. HBx activates the *HULC* promoter through its interactions with the transcription factor cAMP-responsive element-binding protein (CREB) [117]. Interestingly, a genetic variation in *HULC* (rs7763881) is associated with a low-risk susceptibility to HCC in HBV-persistent carriers [118]. Another lncRNA found to be regulated by HBx is *Dreh*, which is downregulated in HBx transgenic mice and in human HBV-related HCC tissues [119]. *Dreh* acts as a tumor suppressor by changing the normal cytoskeleton structure to inhibit tumor metastasis [119].

5   Role of ncRNA in Immune Responses to Virus Infection

Innate immune sensing is a host's first line of defense against invading pathogens. Cellular membrane-bound Toll-like receptors (TLRs) and cytosolic pathogen recognition receptors (PRRs), such as RIG-I and MDA-5, recognize pathogen-associated molecular patterns (PAMPs) present in genomic viral RNA. These viral molecular motifs initiate signaling cascades that culminate in interferon regulatory factor (IRF)-mediated transcription of *IFNβ* gene expression. IFNβ is then secreted from the cell and in an autocrine and paracrine manner binds to type I IFN receptor (IFNAR) expressed on the plasma membrane to induce hundreds of interferon-stimulated genes (ISGs), many with well-known antiviral effector functions. A host must be able to withstand physiological and oxidative stresses triggered by viral infection, such as the effects of NFκB-regulated pro-inflammatory cytokines, including TNF, IL-1, IL-6, and IL-8. Interferon, while the hallmark of innate immunity, is no longer the only major player in initiating and modulating antiviral defenses. As we have discussed, ncRNAs have widespread functions in a variety of cellular processes and the relationship between ncRNA regulation, innate immunity, and inflammatory responses is becoming increasingly clear. Here, we will discuss the current knowledge of ncRNA induction and modulation of innate and adaptive immunity in the context of pathogen–host interactions (Fig. 2).

5.1   *Activation of Innate Immune Responses by miRNAs*

David Baltimore was among the first to study TLR-induced miRNA expression in response to a variety of microbial stimuli. Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is a strong stimulant of innate immune responses and a TLR4 agonist. When used to treat human monocytic THP-1 cells, LPS induces significant differences in miRNA expression [120]. Widespread and transient changes in miRNA expression have also been observed in the lung of mice exposed to LPS, further emphasizing ncRNA changes among transcriptional responses to microbial products. Among the miRNAs profiled in LPS-stimulated THP-1 cells, miR-146a/b, in particular, was significantly induced by pro-inflammatory mediators NFκB, TNF, and IL-1β [120]. Computational analyses identified TRAF6 and IRAK1, downstream kinases of TLR4, as targets for posttranscriptional repression by miR-146a/b based on near perfect complementarity to the miR-146a/b seed sequences (Fig. 2). This early evidence indicated that TLR could induce a potential new class of signaling regulators in response to pathogens and under pro-inflammatory conditions. Indeed, TRL4-responsive miR-146a was largely increased in human airway epithelial cells infected with human H3N2 influenza virus [121]. In a separate study, *Cryptosporidium parvum*, a protozoal species that causes cryptosporidiosis, was found to upregulate TLR4 in
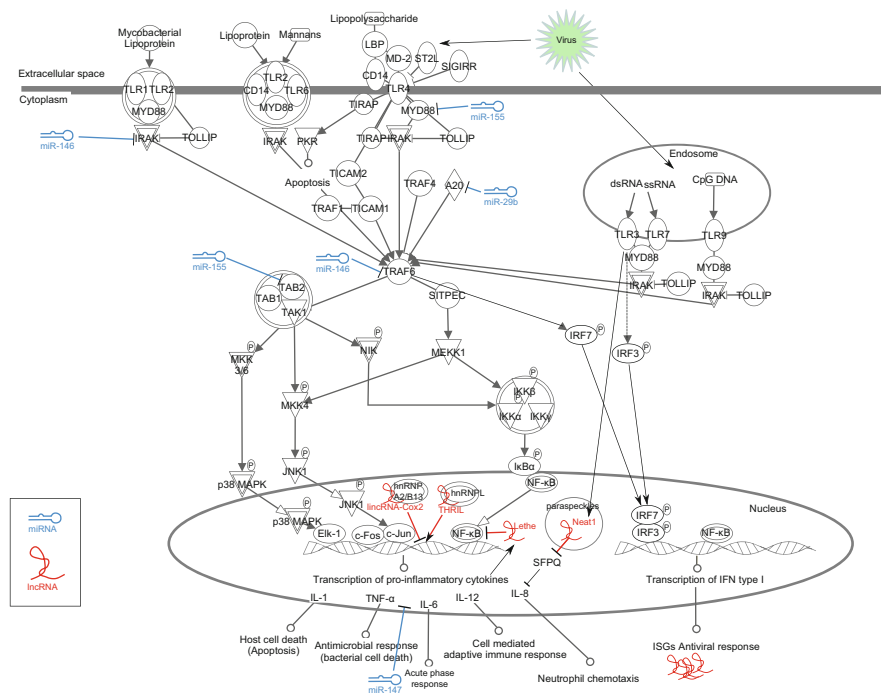
Fig. 2 miRNAs and lncRNAs modulate innate immune response to viral infection. The innate immune-sensing pathway diagram was generated through the use of IPA (Ingenuity® Systems, www.ingenuity.com). MiRNAs are shown in *blue* and lncRNAs are shown in *red*. The cellular targets regulated by these ncRNAs are indicated

infected human biliary epithelial cells, which was concomitant with changes in ncRNA expression, such as decreased *let-7i* expression [122]. While enhancing innate immune signaling, miRNA expression can have the opposite effect on pro-inflammatory mediator production. For example, increases in miRNA expression was accompanied by decreased TNF, KC, a mouse cytokine involved in neutrophil chemotaxis and cell activation, and macrophage inflammatory protein (MIP)-2 expression in vivo, suggesting dual miRNA function in the modulation of host inflammatory responses [123].

In addition to miR-146, changes in the expression levels of several other miRNAs, including miR-155, miR-132, and miR-125b, have been associated with the activation of the innate immune response [124]. Cellular miR-155 is considered a lymphoma-associated oncomir and was recently found to modulate pro-inflammatory activities of different immune cell types, including macrophages [125], monocytes, and dendritic cells (DCs) [126, 127]. In human plasmacytoid DCs, miR-155 expression is increased in an NFκB- and Jnk-dependent manner that resulted in negative regulation of IFNα production [128]. While miR-155 was shown to negatively regulate *IFNα* expression, a separate study argued that

miR-155 promotes type I IFN signaling in antiviral immunity by targeting SOCS1 [129]. Cellular mechanisms aimed at attenuating pro-inflammatory responses also target miR-155-mediated activity, including IL-10, which inhibits miR-155 and acts as a negative regulator of miR-155-mediated pro-inflammatory responses [126]. Taken together, miR-155 not only acts as a regulator of interferon responses but also serves as a target of anti-inflammatory factors that help balance miR-155-mediated enhancement of innate immunity.

Japanese encephalitis virus (JEV) is a mosquito-borne virus and a member of the *Flaviviridae* family that enhances neuroinflammation of the central nervous system. MiR-155 is found to regulate JEV-induced inflammatory responses by targeting Src homology 2-containing inositol phosphatase 1 (SHIP1) 3′ UTR [130]. For example, anti-miR-155 treatment of mice infected with JEV decreases the expression of pro-inflammatory cytokines *TNF*, *MCP-1*, and *IL-6* in the brain. This in turn improves survival and JEV-associated disease, alleviating behavioral symptoms related to body stiffening and hind limb paralysis [130]. Microglial cell activation is a hallmark of neuroinflammation. MiR-29b was found to be significantly upregulated in JEV-infected mouse microglia (BV-2) and primary microglia cells, regulating microglia activation by targeting TNFα-induced protein 3 (TNFAIP3, also known as A20), a negative regulator of NFκB (Fig. 2) [131]. In response to a separate flavivirus, HCV patients with liver fibrosis have downregulated miR-107 and miR-449a expression, with upregulated *CCL2* expression. Both miR-449a and miR-107 were found to regulate IL-6-mediated *CCL2* expression and STAT3 phosphorylation by targeting IL-6R and JAK1 in HCV-infected hepatocytes [132]. Taken together, these studies demonstrate a role for miRNA in regulating inflammation in neurologic and liver diseases caused by flaviviruses.

Inflammatory responses are critical for controlling viral infection. One such mechanism is the inflammasome, an intracellular signaling complex that increases IL-1β when activated. Recently, two miRNAs encoded by EBV with sequence homology to miR-223 were shown to target the miR-223 binding site in the NLRP3 3′ UTR and inhibit inflammasome activity [133]. This demonstrates the diverse strategies of virally encoded miRNAs in mimicking cellular miRNA function regulating inflammatory pathways. Endogenous inflammatory mediators called damage-associated molecular patterns (DAMPs) have been suggested to modulate immune responses and inflammation, though their role in the context of viral infection is poorly understood. DAMP molecule, S100A9 (also known as Calgranulin B or MRP-14), enhances inflammation during acute influenza infection by acting as a non-PAMP activator via the TLR4-MyD88 pathway [134]. Interestingly, a new miRNA class known as damage-associated molecular pattern molecule-induced miRNAs (DAMPmiRs) was recently identified in human peripheral blood mononuclear cells [135]. Future studies will likely unveil the role of DAMPmiRs in virus-induced inflammatory responses.

5.2   Regulation of Inflammatory and Innate Immune Responses by lncRNA

Many studies have shown that lncRNAs have central roles in the control of gene expression during cellullar differentiation in the development of diverse organs and tissue types (reviewed in [136]). In addition to their roles in hematopoiesis and leukemogenesis [137–139], many lncRNAs are involved in immune cell activation (reviewed in [140]). Moreover, some lncRNAs modulate inflammatory and innate immune gene expression following the activation of some TLRs.

Similar to miRNAs, lncRNAs are expressed in response to TLR agonists. In mouse dendritic cells, 20 lncRNAs showed marked upregulation after stimulation with LPS including lincRNA-*Cox2*, which was not upregulated in TLR3-stimulated cells [82]. LincRNA-*Cox2* was also upregulated in mouse macrophages stimulated with TLR2 ligand and following *Listeria monocytogenes* infection [141]. Silencing and overexpression of lincRNA-*Cox2* revealed that the lncRNA regulates distinct classes of immune genes both basally and after TLR stimulation [141]. Using a custom microarray, 159 lncRNAs were found to be differentially expressed following TLR2-stimulation of human macrophages, including *THRIL* (also known as linc1992), which was found to be downregulated [142]. *THRIL* and lincRNA-*Cox2* both interact with different heterogeneous nuclear ribonucleoproteins (hnRNPs) to bind immune gene promoters. LincRNA-*Cox2* forms a complex with hnRNP-A/B and hnRNP-A2/B13 to repress the transcription of some immune genes including chemokine *CCL5* gene [141], while *THRIL* binds hnRNPL to stimulate *TNFα* gene transcription (Fig. 2) [142].

Aside from these TLR2/TLR4-mediated lncRNA regulations, the lncRNA *NEAT1* is induced after TLR3 stimulation and in response to influenza virus, herpes simplex virus 1 (HSV-1) [104], and HIV [103]. Upon infection, viruses detected through TLR3 activate p38 MAPK pathway which leads to *NEAT1* induction (Fig. 2). This relocates the transcriptional repressor, SFPQ, from the promoter region of antiviral genes into paraspeckles, leading to the transcriptional activation of antiviral genes such as *IL-8* (Fig. 2) [104]. Finally, numerous lncRNAs can be induced in response to cytokine stimulation, such as IFNβ [85, 88] and TNFα [143]. Among the 166 mouse lncRNAs induced by TNFα, a pseudogene transcribed into lncRNA named *Lethe* is also expressed in response to IL-1β or glucocorticoid receptor agonist, but it is unresponsive to TLR1-7 agonists. *Lethe* is directly induced by NFκB signaling and acts as a negative feedback inhibitor of NFκB by binding to RelA.

5.3   Regulation of Adaptive Responses by miRNA During Viral Infection

The inflammatory response has been linked to aberrant allergen-specific CD4+ T-helper 2 (T$_H$2) cell function and the recruitment and activation of eosinophils and

mast cells in the airways. T_H2 cell-induced eosinophilia and airway hyper-responsiveness (AHR), as well as the secretion of a range of cytokines, including IL-4, IL-5, IL-9, IL-10, and IL-13, are hallmark features of allergic asthma, of which the TLR4 signaling pathway is central to the progression of allergic airway disease, as mice deficient in TLR4 signaling have been shown to have an attenuated allergic phenotype [144]. Single miRNAS can have profound effects on the development of pulmonary inflammation. For example, antagonism of miR-126 suppresses the effector function of T_H2 cells, including the recruitment of eosinophils and neutrophils into the airways and the overproduction of airway mucus [145].

MiR-155 is upregulated in primary effector and effector memory CD8+ T cells and it is required for lymphocyte responses and effector T cell memory against influenza virus and *Listeria monocytogenes*. Antiviral CD8+ T cell responses and viral clearance were impaired in miR-155 knockout (KO) mice that exhibited reduced primary and memory responses, whereas CD8+ T cell responses were much more robust when miR-155 was overexpressed in infected mice [146]. MiR-155 has also been investigated for its role in cytotoxic T cell function in response to lymphocytic choriomeningitis virus (LCMV). Mice lacking miR-155 are deficient in primary CD8+ T cell responses following LCMV infection due to impaired Akt signaling that impacts cell survival. After infection, there were reduced numbers of IFNγ-producing T cells found in miR-155 KO spleens compared to wild-type (WT) spleens, as well as differential CD8+ T cell responses in the spleen between WT and KO mice [147].

5.4   Regulation of Adaptive Responses by lncRNA During Viral Infection

Expression of ncRNAs during T cell development was observed almost 30 years ago, with the transcription of the T early alpha (*TEA*) noncoding transcript in the T-cell receptor alpha chain locus [148] required for V(D)J recombination assembling the variable regions of T cell receptors [149]. Several lncRNAs were also found expressed in activated CD4+ T cells, including *NTT* [150], and the proto-oncogene, *BIC* [151].

More recently, systematic characterization of lncRNAs identified over 1,000 lncRNAs dynamically expressed during CD8$^+$ T cell differentiation and activation after exposure to viral antigens [152]. Further exploring lncRNAs involved in T cell function, Hu et al. identified 1,524 regions encoding for lncRNA that were expressed at various developmental and differentiation stages of T cells [84]. The expression of most of them is controlled by T cell-specific transcription factors. Among these lncRNAs, LincR-*Ccr2-5′*AS was specifically expressed in T_H2 cells and its expression was positively correlated with genes involved in chemokine signaling. Silencing of lincR-*Ccr2-5′*AS by shRNA led to deregulation of many genes, including lower expression of the neighboring *Ccr1*, *Ccr2*, *Ccr3*, and *Ccr5* genes. This resulted in reduced migration of T_H2 cells to the lung. While the

mechanism of gene regulation by lincR-*Ccr2-5'*AS is still being elucidated, it is known to be distinct from the modulation of chromatin accessibility or recruitment of RNA polymerase II, a common mechanism for lncRNAs to regulate gene expression [153].

One of the most studied lncRNA in viral infection, *Tmevpg1* (also named *NeST*), was initially discovered for its role in Theiler's virus persistence [154, 155]. Theiler's murine encephalomyelitis virus (TMEV or Theiler's virus) is a picornavirus that causes acute infections in mice. In some mouse strains, such as SJL/J, TMEV causes persistent infection of the spinal cord followed by a late chronic demyelinating disease similar to multiple sclerosis in humans. TMEV infection is mainly controlled by the *H2D* class I gene; however, two other susceptibility loci were mapped on chromosome 10 close to the *IFNγ* locus and named *Tmevp2* and *Tmevp3* [156]. The lncRNA *Tmevpg1* was identified in the *Tmevp3* locus [154] and was expressed in unstimulated T cells and in CNS-infiltrating immune cells of resistant B10.S mice after TMEV infection [155]. While Vigneau et al. initially hypothesized that *Tmevpg1* may downregulate the expression of *IFNγ*, *Tmevpg1* was later found to positively regulate the transcription of *IFNγ* in cooperation with the transcription factor T-bet in CD4+ T_H1 T cells [157]. Recently, Gomez et al. showed that transgenic expression of SJL/J-derived *Tmevpg1* allele in B10. S mice prevents the clearance of TMEV, yet confers resistance to lethal infection with *Salmonella enterica* serotype Typhimurium [158]. Therefore, they renamed *Tmevpg1* as *NeST* for "Nettoie *Salmonella* not TMEV" (clear *Salmonella* not TMEV). The SJL/J-derived *NeST* allele also conferred increased resistance to the lethal inflammatory disease caused by LPS injection. In contrast to the previous report of Vigneau et al. [155], *NeST* RNA was undetectable in T cells from B10.S, but it was expressed in SJL/J mice and its RNA level positively correlated with *IFNγ* transcription. *NeST* was shown to interact with the subunit WDR5 of the H3K4 methyltransferase complex that catalyzes the trimethylation of histone H3 at lysine 4, a mark of active gene expression [158]. Increased *NeST* RNA abundance in CD8+ T cells was associated with increased H3K4 trimethylation at the *IFNγ* locus and increased IFNγ synthesis in splenic tissue. A proposed mechanism is that *NeST* recruits histone H3 lysine 4 methyltransferases to the *IFNγ* gene locus, enhancing IFNγ expression in key T cell subsets. However, the link between NeST-mediated IFN regulation and its opposite effects on TMEV and *Salmonella* control remains unclear. In particular, whether *NeST* may regulate the chromatin state of other genes with key roles in the immune response to pathogens was not tested. It would be interesting to systematically characterize *NeST* functions using systems biology approaches [159].

6 Conclusion

The noncoding transcriptome has been increasingly implicated in regulating human development and disease over the past decade, with several reports suggesting that ncRNAs have enormous clinical potential. MiRNAs are being developed as serum

biomarkers in cancer detection and diagnosis, and their usefulness toward a variety of clinical diseases is being explored. Cell-free miRNAs have been detected in the blood of patients with diffuse large B-cell lymphoma [160], Duchenne muscular dystrophy [161], pediatric Crohn disease [162], cardiovascular diseases (reviewed in [163]), and HBV-positive HCC cases [164]. For example, circulating miR-146a and miR-223 were significantly decreased in patients with sepsis compared to normal control patients. These candidate biomarkers could be used in conjunction with well-known biomarkers of acute systemic inflammation, such as C-reactive protein (CRP) [165]. MiR-122 is highly expressed in the liver, and serum miR-122 has been found to correlate with virologic responses to pegylated IFN therapy in chronic HCV [166], liver injury in patients with chronic HBV [167, 168], and HCC [169]. Therapeutic silencing of miR-122 in nonhuman primates with chronic HCV has been assessed and shown to suppress viremia [170]. In clinical settings, this would be a vast improvement to the current standard of care that combines pegylated IFNα with ribavirin, which is effective in only 50 % of patients and associated with serious side effects. Considering the therapeutic potential of miR-122, Regulus Therapeutics Inc. and GlaxoSmithKline are currently developing miRNA drugs linked to inflammatory diseases for commercialization. LncRNAs, such as *HBx-LINE1*, may also hold great promise as potential biomarkers for diagnosis and prognosis of viral infection [12]. Other host lncRNAs, such as *MALAT1*, could also be used as prognosis biomarkers for several cancers, independently of viral infection. Finally, long noncoding mitochondrial RNAs (ncmtRNAs) were recently reported as deregulated following human papillomavirus (HPV) infection [171]. The expression profile of these transcripts allows researchers to distinguish between normal, pre-tumoral, and cancer cells. One of these transcripts, SncmtRNA-1, has been characterized as a regulator of cell cycle progression, while two others, ASncmtRNA-1 and 2, have been suggested to act as three tumor suppressors. Expression of SncmtRNA-2 might contribute to the screening of early cervical intraepithelial lesions [172].

To conclude, novel sequencing technologies and computational methods have widely expanded the landscape of the mammalian transcriptome. NcRNAs have emerged as key players regulating various biological processes, including viral infection. Among ncRNAs, many miRNAs and lncRNAs have been identified as deregulated following viral infection. Only a few lncRNAs have been functionally characterized, but it is clear this class of ncRNAs is involved in a large variety of biological processes, similar to the ubiquitous importance of miRNAs. Virally encoded and host-derived miRNAs and lncRNAs have been linked to the virulence and pathogenesis of several different DNA and RNA viruses. They regulate viral replication and pathogenesis and modulate innate and adaptive immune responses to viral infection. Research on ncRNAs opens new avenues toward novel therapy and diagnostic tools, as well as development of novel paradigms about transcriptome regulation of biological systems. Most recent advances include the characterization of circular RNAs [173], RNAs acting as competing endogenous RNAs (ceRNAs) or natural microRNA sponges [174], and mRNA with noncoding functions such as *HBx-LINE* [12]. Now, the challenge is to understand their

functions and how these diverse RNAs interact together to form a large interconnected transcriptional network that can shape the outcome of viral infection.

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