Review Article

Long noncoding RNAs and viral infection: promising molecular markers?

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Abstract: Studies both *in vitro* and *in vivo* suggest that long noncoding RNAs (lncRNAs) are markers of viral infection. lncRNA expression patterns change when host cells are infected with a virus. Conversely, viral lncRNAs also regulate the gene expression of host cells, facilitating the replication of the virus itself and its escape from immune surveillance. Combined with clinical detection methods, lncRNAs are potential clinical biomarkers and may help in the diagnosis of virus-related diseases.

Keywords: Long noncoding RNAs (lncRNAs); virus; biomarker

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Introduction

For years, long noncoding RNAs (lncRNAs) were considered to be transcriptional noise. When viruses invade host cells, we used to think that they replicate and inhibit host antiviral responses through pathways induced by protein complexes. Nowadays, however, we know that various small RNAs in serum, such as microRNAs and lncRNAs, can be used as markers to determine the presence of viral infection and to obtain reliable laboratory data for differential diagnoses of virus-related diseases.

Meet lncRNAs

It was once believed that mRNAs make up the majority of the transcriptome. However, with the development of high-throughput sequencing and ENCODE (1), researchers realized that noncoding RNAs (ncRNAs) are also vital components within cell membranes. NcRNAs originate from transcription from the genome. According to their length, ncRNAs are classified into two subgroups: small ncRNAs and lncRNAs with more than 200 base pairs. LncRNAs are transcribed by RNA polymerase II and are found in nuclear and cytosolic fractions. They take part in chromatin transport, stem cell maintenance, and cell differentiation. According to recent reports, their biological functions are as follows: (I) the upstream promoter region of protein-encoding genes transcription interferes the expression of downstream genes; (II) affecting the expression of downstream genes by inhibiting RNA polymerase II or mediating chromatin remodeling and histone modifications; (III) forming complementary double-stranded chains with transcripts that encode proteins to interfere with mRNA splicing, and then inducing the expression of different splicing variants; (IV) forming complementary double-stranded chains with transcripts that encode proteins to produce endogenous siRNA under the action of the Dicer enzyme; (V) regulating the activity of proteins by combining with specific partners; (VI) forming nucleic acid-protein complexes with proteins as structural components; (VII) binding to specific proteins to alter their cellular location; and (VIII) acting as precursor molecules of small RNAs, such as miRNAs.

Reverse transcription polymerase chain reaction (RT-PCR), fluorescence *in situ* hybridization (FISH), and microarray are currently employed to detect lncRNAs. Iizuka (2) studied differences in the profiles of lncRNA expression between hepatocellular carcinoma and adjacent healthy tissues. The results showed that lncRNAs with altered expression in HCC included high expression in HCC (*HEIH*), highly upregulated in liver cancer (*HULC*), HBx-long interspersed nuclear element 1 (*HBx-LINE1*), *H19*, maternally expressed imprinted gene 3, and downregulated expression by HBx (*DREH*). A higher level of *HULC* in HBV-related HCC was reported elsewhere and confirmed to be a convenient diagnostic biomarker following amplification (3). In addition, Wang (4) reported that *HULC* expression levels in hepatocellular carcinoma and hepatocarcinoma cell lines were (7.6±3.1) $\times 10^5$ and (5.6±3.2) $\times 10^6$ copies/L, respectively, which were significantly higher than those of other cancer and paracarcinoma tissues (<1.8×10^3 copies/L). The minimum detection limit of RT-PCR to detect *HULC* is 1.8×10^3 copies/L, with a linear range of 1.8×10^3 to 4.6×10^9 copies/L [coefficient of variation (CV) <2.0%, batch CV <8.0%]. Recently, Panomics QuantiGene Plex technology developed new assays that enable the simultaneous detection and quantification of lncRNAs of 3–80 nucleotides in length in cultured cells, whole blood samples, and tissue samples without reverse transcription or PCR.

lncRNAs, viruses, and immune responses

Viruses have developed various strategies to fight against the human immune system and to increase the efficiency of their self-replication. Some of these strategies are implemented through lncRNAs.

lncRNA expression of host cells by viral infection

Viral infection induces the abnormal expression of lncRNAs in host cells. The first lncRNA screening in mice and mouse embryonic fibroblasts in response to influenza A virus infection revealed that hundreds of potential lncRNAs were differentially expressed. The study (5) further analyzed the expression levels of lncRNAs in human A549 cells after infection with the A/Victoria/3/75 (H3N2) influenza virus. Total nonribosomal RNA was isolated 6 h postinfection and analyzed by deep sequencing, which revealed that 141 lncRNAs were upregulated. Most of them had not been identified so far. The others have been implicated in a variety of biological processes, including cell differentiation and cancer development.

In a comparison with control cells, X protein (hepatitis B virus x protein, HBx) was found to significantly downregulate the tumor suppressor lncRNA *DREH* (downregulated expression by HBx) in HBV-infected cultured cells or transgenic mice. *DREH* was typically low in malignant tissues when compared with its level in patient-matched noncancerous hepatic tissues. The role of HBx in *DREH* downregulation was further confirmed by transfecting murine hepatic cell lines with plasmids encoding the viral protein (6) and engrafting nude mice with murine cells expressing *DREH*. *DREH* inhibition disrupted cell proliferation, apoptosis, and migration. In another study, Braconi (7) applied a microarray and revealed that 174 lncRNAs were highly expressed and 712 lncRNAs were downregulated in HCC cells, of which *HULC* and *HEIH* were strongly involved in HBV-induced HCC. HBx binds to the *HULC* promoter by interacting with the transcription factor CREB (cAMP-response element binding protein) and induces *HULC* expression, which inhibits the tumor suppressor *P18* and promotes cancer cell proliferation (8-10). On the other hand, *HEIH* recruits PRC2 (polycomb repressive complex 2) through the enhancer of zeste homolog 2 (*EZH2*). Together, they inhibit *EZH2* target gene expression and arrest cells in the G_0/G_1 phase.

Viral lncRNAs influence host cell function

During infection, viruses maintain themselves in a latent or replicative state by encoding their own lncRNAs. At present, we have some understanding of the specific functions of viral lncRNAs in the process of viral infection (*Table 1*) (23). For example, β2.7 *RNA* is a highly conserved lncRNA with 2,700 base pairs transcribed by human cytomegalovirus. It localizes in the cytoplasm of host cells and accounts for more than 20% of the total viral transcripts (11). Along with mitochondrial enzyme complex I, β2.7 *RNA* stabilizes mitochondrial membrane potential and ensures the ATP production of host cells, in order to avoid stress cascade responses, inhibit host cell apoptosis, and promote viral survival (12).

Viral lncRNAs are epigenetic regulatory factors. For example, during the process of human immunodeficiency virus (HIV) infection, the HIV genome encodes antisense RNA transcripts to suppress the expression of virus-encoding genes (24). Recently, it was found that the 5'-long terminal repeat (LTR) region of the HIV virus

Table 1 Viral lncRNA biofunction

lncRNA name	Virus	Functional characteristics	References
β2.7	HCMV	Combines with the mitochondrial enzyme complex and prevents apoptosis of virus-infected cells to maintain the production of energy by mitochondria	(11-13)
ASPRO5	HIV	Reverse-transcribed in the 5'-LTR region of HIV; inhibits HIV promoter activity; silences viral gene expression	(14)
HBx-LINE1	HBV	When the HBV genome is inserted into the short arm of chromosome 8, this chimeric transcript increases the malignancy of the tumor and promotes cell migration	(15)
EBERs	EBV	Transcribed by RNA polymerase III; contain a 5'-polyA end; regulate the innate immune system of host cells; highly expressed in latent EBV	(16)
HSURs	HSV	Include HSUR1 and HSUR2; induce miR-27 degradation and gene expression of T cells	(17,18)
VAs	HAdV	Increased during viral replication; produce the RNA silencing complex	(19-22)

encodes an antisense lncRNA named *ASPRO5*. It recruits DNA methyltransferase 3A (DNMT3a), histone deacetylase HDAC-1 (histone deacetylase 1), and histone methyltransferase EZH2 to the 5'-noncoding region of the virus to form a heterochromatin structure like histone H3 lysine 9 dimethylation (H3K9me2) and trimethyl histone H3K27 (H3K27me3), followed by histone deacetylation and inhibition of virus transcriptional activity. Silencing *ASPRO5* activates HIV gene expression (14).

Chimeric lncRNAs

Intensive studies of HCC led to the identification of transcripts in which human and viral sequences are fused (25). This is unsurprising as the HBV genome can integrate into the host genome. Integration preferentially occurs within repetitive sequences, such as LINEs. Results have shown that 23.3% of HCCs express chimeras between the HBx protein and host LINE1 sequences. These chimeric transcripts, named HBx-LINE1, have oncogenic effects as they activate Wnt signaling pathways. Conflicting results have been obtained about whether viruses can benefit from the transcription of chimeric lncRNAs (26).

Immune responses and lncRNAs

In recent years, the roles of lncRNAs in the development of immune cells and the regulation of immune responses have been discussed. The innate immune response is the first line of defense of the body against invading pathogens, mainly implemented by innate immune cells including monocytes/macrophages, dendritic cells (DCs), granulocytes, and natural killer cells. Wang (27) analyzed the lncRNA expression profile of DCs differentiating from monocytes and DCs that had undergone maturation induced by lipopolysaccharide. They found that the expression levels of 76 lncRNAs were significantly changed (>20 times), among which lnc-DC was highly expressed in mature DCs. It facilitates the expression of antigen-presenting molecules and costimulatory molecules on the surface of mature DCs, and stimulates IL-12 secretion.

TMEVPG1 (NeST, Ifng-AS1) was initially identified as an lncRNA transcript in the context of Theiler's virus infection. Mice lacking *TMEVPG1* were unable to control intracranial viral infection. *TMEVPG1* is absent from the central nervous system (CNS). However, when Theiler's virus infected resistant B10.S mice, it began to be expressed in CNS-infiltrating immune cells such as macrophages, microglia, and astrocytes. Similar results for *TMEVPG1* were observed in human NK cells and $CD4^+$ and $CD8^+$ T lymphocytes (28). Both Temvpg1 and its human ortholog *TMEVPG1* are localized in a cluster of cytokine genes including IL-10 homologs and IFN-γ. Research (29) found that interfering with *TMEVPG1* expression significantly inhibited IFN-γ secretion in mouse Th1 cells. The combined action of *TMEVPG1* and T-bet upregulated IFN-γ level, while the overexpression of *TMEVPG1* alone did not, suggesting that *TMEVPG1* regulates IFN-γ gene transcription by interacting with other factors.

Prospects of clinical application

lncRNAs are a new hotspot in clinical research. They regulate the expression of genes through different molecular biological

mechanisms, are involved in multiple disease-related signaling pathways, and play an important role in disease prognosis. Disease is the result of multiple molecular dysfunctions in cells and circumstances around them. Protein-coding genes are only one part of the interaction network in the pathophysiological processes of diseases. The identification of more lncRNAs should shed light on pathophysiological mechanisms from an RNA perspective. According to research data, the sensitivity and specificity of lncRNA detection assays are higher than those of assays of protein in body fluid. As new promising biomarkers, lncRNAs thus have broad potential for clinical applications. Although microRNAs are the most dazzling stars at present, lncRNA should become a brighter young member of the revealed complex of molecular networks related to viral infection.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jlpm.2017.05.13). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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