Roles of IncRNAs in the transcription regulation of HIV-1

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Abstract
Long noncoding RNAs (IncRNAs) is a class of RNA molecules that are more than 200bp but cannot be translated into proteins. More and more studies have proved that IncRNA plays a crucial role in various biological functions and disease processes, including virus infection. It's worth noting that studies have also shown that IncRNAs play an essential role in the pathogenesis of human immunodeficiency virus 1 (HIV-1), one of the lethal virus that can destroy immune system. Although IncRNA-mediated gene regulation involves a variety of mechanisms, such as transcription regulation, translation regulation, protein modification, and the formation of RNA-protein complexes, in this review, we primarily focus on the role of IncRNAs in HIV-1 transcription regulation, which is one of the most important mechanisms that control the activation and development of HIV-1. This review also briefly summarizes the latest research progress of IncRNAs related to HIV-1 infection and its potential application in HIV-1 therapy. Although there are antiretroviral drugs that interfere with the function of HIV-1 virus-encoded proteins, this treatment for the HIV-1 virus is limited by its ability to produce drug resistance. Hence, a further understanding of HIV-1 transcription regulation by IncRNAs might help develop non-traditional antiviral therapy strategies.

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RNAs are conventionally regarded as the bridge between DNA and protein, but transcriptome analysis shows that only 2% of the genome is responsible for the transcription of protein coding sequences and that more than 70% of genome regions are transcribed into noncoding RNAs (ncRNAs) [1]. Over the last decade, there has been an increased interest in the roles that ncRNAs play in various physiological processes, and dysfunction may impact several pathologies, particularly viral infection and antiviral responses. Among them, human immunodeficiency virus 1 (HIV-1) represents one of the most significant retroviruses, with a genome approximately 9.8 kb in length consisting of 9 partially overlapping genes [2-4]. An increasing number of studies have pointed out that long noncoding RNAs (lncRNAs) may represent targets of choice for HIV latency reversal [54,59,62,78,79]. The transcriptional regulation of HIV-1 is crucial in the activation and development of HIV-1, so research on its transcriptional regulation by potential lncRNAs deserves more attention.

Hence, this review highlights the role of lncRNAs in HIV transcription regulation and further provides a discussion of the potential clinical applications and challenges. Notably, although most studies on HIV focus on HIV-1, this review uniformly refers to the virus as ‘HIV’.

**The possible mechanisms by which IncRNAs regulate gene expression**

Most lncRNAs greater than 200 bp in length are byproducts of RNA polymerase II-mediated transcription, usually 5’ capped, spliced and polyadenylated. Structurally, lncRNAs localize preferentially to the nucleus, remaining more tissue-specific, underexpressed and less evolutionarily conserved than mRNAs [5]. Mechanistically, lncRNAs have the ability to regulate gene expression by interacting with DNAs, RNAs, or proteins, either positively or negatively. At the transcriptional level, lncRNAs are apt to regulate nearby genes in cis and distant genes in trans [6,7]. Here, various regulatory functional mechanisms have been shown in Fig. 1, involving epigenetic modification, regulation of transcriptional or posttranscriptional gene expression and competing endogenous RNAs (ceRNAs) [8-11].

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Fig. 1 Four mechanisms of IncRNA in the regulation of gene expression. (A) Epigenetic modification: LncRNA can bind to one or more chromatin modification complexes, recruit the complexes to specific genome loci, where they acetylate or methylate lysine residues of histone H3 as well as methylate DNA to activate or silence transcription. (B) Transcription regulation: LncRNA can bind to transcription factors, ribonucleoproteins (RNP), or specific transport factors to enhance or inhibit gene expression. LncRNAs can also directly bind to Pol II to inhibit transcription. The formation of lncRNA-DNA structure can also inhibit the pre-initiation complex assembly, thus inhibiting gene expression. (C) Post-transcriptional regulation: An antisense lncRNA can bind to the 5’ splice site of an intron in the 5’ UTR of the zinc finger Hox mRNA Zeb2 and retain critical cis-elements internal ribosome entry site (IRES) in the intron, the translation machinery can then recognize and bind the IRES in the retained intron, thus promoting mRNA translation. (D) CeRNAs: LncRNAs can compete with mRNAs for binding microRNAs (miRNAs), which can block miRNA-induced silence, thus increasing the translation of mRNA. LncRNAs can also be used as miRNA bait to isolate miRNAs from their mRNA target.
DNA methylation and histone modification can alter the state of chromatin, resulting in transcriptional activation or silencing. In this case, lncRNAs recruit chromatin remodeling components to specific genome loci and reprogram the state of chromatin to silence or activate transcription (Fig. 1A). For instance, HOX transcript antisense intergenic RNA (HOTAIR), a lncRNA expressed from a developing HOXC locus, is regarded as a scaffold to recruit polycomb repressive complex 2 (PRC2) and lysine specific demethylase 1 (LSD1) in trans. This recruiting contributes to histone H3 lysine 27 methylation associated with transcriptional inhibition and H3K4me2 demethylation associated with transcriptional activation [12]. Additionally, lncRNAs can regulate the expression of neighboring genes, especially in the process of imprinting. LncRNA Air is imprinted and expressed only on the paternal allele and recruits histone H3 lysine 9 (H3K9) histone methyltransferase G9a to the promoter of Slc22a3, thereby leading to targeted H3K9 methylation allelic silencing [13].

Transcription regulation

Speculatively acting to repressively regulate gene transcription, lncRNAs may act as decoys by binding their targets (Fig. 1B). This activity is regarded as a mechanism of competitive regulation between lncRNAs and other molecules, aiming to exert an effect on the same molecular targets. Emerging evidence shows that IncRNA Lethe is directly induced by nuclear factor-kappa B (NF-κB) after stimulation with the glucocorticoid receptor dexamethasone or tumor necrosis factor-α (TNF-α). Alternatively, the IncRNA Lethe interacting with RelA–RelA homodimers inhibits binding to other NF-κB response elements and target gene activation. Consequently, this interaction leads to a decrease in the expression of downstream effectors, such as interleukin-6, superoxide dismutase 2, interleukin-8, and NF-κB [14].

Furthermore, IncRNA THRIL and IncRNA-COX2 regulate the transcription of TNF-α and chemokine (C-C motif) ligand 5 by forming a complex with heterogeneous ribonucleoprotein (hnRNP) subtypes [15,16].

Post-transcription regulation and competing endogenous RNA (ceRNAs)

LncRNAs are also involved in the posttranscriptional regulatory network. For example, Zeb2 (also called Sip1) antisense RNA can bind to the 5' splice site of an intron in the 5' UTR of the zinc finger Hox mRNA Zeb2 and then retain critical cis-element internal ribosome entry sites (IRESs) in mRNA by forming RNA duplexes, thus resulting in efficient mRNA translation (Fig. 1C) [17]. In addition, ceRNAs compete with mRNAs for microRNA (miRNAs) with shared miRNA response elements (MREs) and act as regulators of miRNAs by affecting the availability of miRNAs [18] (Fig. 1D). Recently, it has been proposed that IncRNAs act as miRNA sponges by sharing a common MRE and inhibiting the targeting activity of miRNAs on mRNAs (Fig. 1D). LINC-MD1, a cytoplasmic lncRNA expressed during myoblast differentiation, acts as a ceRNA for miR-133 and miR-135 to regulate myocyte enhancer factor 2C, mastermind-like 1, and myoblast differentiation [19].

**Epigenetic modification**

HIV is a retrovirus that was first isolated in 1983 [20,21]. Each virion contains two copies of the RNA genome. When the RNA genome is reverse transcribed into cDNA, it is integrated into the human genome for expression. The HIV genome is 9.8 kb in length and consists of 9 partially overlapping genes [2–4] (Fig. 2). Intricate splicing patterns lead to the production of more than 40 unique HIV mRNA overlapping genes (Fig. 2). The group-specific antigen (gag), polymerase (pol), and envelope (env) genes mainly encode structural proteins or enzymes. The p55
Gag protein precursor is processed into p17 matrix protein (MA), p24 capsid (CA), and p7 nucleic acid binding (NC) structural proteins necessary for viral particle assembly [22]. P160 Gag-Pol protein precursor is processed into reverse transcriptase, protease (PR), and integrase (IN), which play a crucial role in the viral replication cycle [23]. Both the Gag and Gag-Pol polyprotein precursors are cleaved into their subunits by viral PR (Fig. 2). The gp160 Env protein is cleaved by cyto- proteases into gp120 and transmembrane gp41 subunits of the env glycoprotein (Fig. 2). This cleaving is necessary for binding to the primary CD4 receptor on host cells [24]. Gp120 initially binds to the cellular CD4 receptor, resulting in conformational changes in gp120, enabling it to bind to coreceptor such as CCR5 or CXCR4 [25]. Coreceptor binding then triggers the interaction between the gp120 transmembrane subunit and the host cell membrane, resulting in the fusion of the cell membrane and the virus membrane [26]. Finally, the virus core containing the virus genome is released into the cell [27]. Transactivator of transcription (Tat) and regulatory factor of viral gene expression (rev) encode two regulatory proteins. Tat is a multispeckled transcript synthesized early in the life cycle of the virus. It binds to the transactivation response (TAR) element in the R domain of the S’ long terminal repeat (LTR) and regulates transcription. TAR RNA recruits positive transcription elongation factor b (P-TEFb) to the promoter proximal paused RNA polymerase II. P-TEFb is an indispensable host cellular factor that manipulates productive transcription elongation [28]. Rev is translated from a fully spliced early transcript and is responsible for mediating the transport of transcripts from the nucleus to the cytoplasm. Unspliced transcripts, including genomic RNA, Gag-Pol precursors, and incompletely spliced mRNAs that encode Env, Vif (viral infectivity factor), Vpr (viral protein R), and Vpu (viral protein U), require interplay between the Rev protein and the Rev responsive element in these transcripts for nuclear export [29]. Except for these five genes, HIV has four accessory genes: Nef (negative factor), Vif, Vpr, and Vpu, which are not required for HIV replication [30, 31]. The protein-coding sequence is flanked by S’ and 3’ LTRs. Each LTR consists of three domains, namely, the U3 (untranslated 3’), R (repeat), and U5 (untranslated 5’) domains. The U3 region contains the essential promoter sequence, enhancer sequence, and multiple transcription factor binding sites, which is also the leading region for the study of HIV transcription regulation [32, 33].

Transcription initiation occurs at the first nucleotide in the R region, which also encodes a Tat-binding TAR with a stem–loop structure that regulates transcription. As mentioned before, it is obvious that HIV has complex splicing. In addition, restricted by a relatively simple genome of just 8.9 kb and 9 genes, the virus has evolved sophisticated ways to hijack host-cell machinery for its own survival. At present, a series of host-dependent factors critical to the infection cycle of HIV have been identified. For examples, NF-κB can promote HIV transcription; p21-activated kinase 1 allows for more efficient viral replication; Cyclin T1 improves HIV replication [34, 35]; barrier-to-autointegration factor 1 is required for the association of viral cDNA with emerin and for the ability of emerin to support virus infection; emerin is necessary for chromatin engagement by viral cDNA before integration [36]; human lens epithelium-derived growth factor/transcription coactivator p75 is essential for nuclear and chromosomal targeting of HIV-1 Integrase [37] and Furin regulates HIV infection via intracellular proteolytic cleavage of gp160 [35, 38]. Currently, most studies on HIV mainly focus on proteins, and elucidating HIV transcription and replication through an in-depth understanding of lncRNAs in these aspects is a priority. Such information can provide more theoretical sources on how HIV enters and maintains latency.

**LncRNAs in the transcriptional regulation of HIV**

A substantial number of reports indicate that ncRNAs are vital elements in HIV-related transcriptional regulatory networks. Among them, lncRNAs have become a research focal point in recent years. A case of primary HIV infection in vitro was analyzed by a transcriptome map to study the expression of lncRNAs in different stages of HIV replication, and it suggested that lncRNAs may be a target for controlling HIV replication [39]. With the advancement of research, the importance of lncRNAs to HIV replication is self-evident. They play a crucial role in the transcriptional regulation of HIV by regulating different cellular signaling pathways. In this section, we summarize and discuss the possible mechanism and applications of lncRNAs related to HIV transcription regulation.

**7SK**

Small nucleus noncoding RNA (snRNA) 7SK is a highly conserved ncRNA with 331 nucleotides in animals that might be derived from some pseudogenes and transcribed by RNA polymerase III (Pol III) [40]. 7SK interacts with methyl phospho-capping enzyme (MePCE), hexamethylene bisacetamide-induced proteins 1 and 2 (HEXIM1 and HEXIM2), and La-associated protein 7 (Larp7) to form ribonucleoprotein (RNP) complexes. MePCE can add a γ-monophosphate to protect the 3’ end of 7SK, HEXIM1 and HEXIM2 can directly interact with the active site in cyclin-dependent kinase 9 (CDK9) through the PYNT motif and inhibit the kinase activity of P-TEFb, and Larp7 can bind and protect the 3’ terminal U residues as well as a C-terminal xRRM that binds to the 3’ stem–loop structure of 7SK and is required for 7SK small nuclear ribonucleoprotein (snRNP) stability [50, 52]. P-TEFb is composed of a CDK9/cyclinT1 (CycT1) heterodimer, which functions as the general transcription elongation factor and can be recruited by HIV-1 Tat (transactivating protein). P-TEFb activates transcription by phosphorylating RNA polymerase (Pol) II, leading to the formation of a process extension complex. Regulating the activity of P-TEFb is the critical function of 7SK ncRNAs [41-44]. 7SK inhibits general transcriptional activity by sequestering P-TEFb in an inactive form in vivo and in vitro (Fig. 3A). Specifically, 7SK acts as a scaffold to assemble LARP7, MePCE, HEXIM1 and P-TEFb to form a complex to sequester P-TEFb in the catalyzed inactivated 7SK snRNP, thus inhibiting the cyclin-dependent kinase activity of P-TEFb and the transcription of HIV mediated by P-TEFb. Details on the transcriptional regulation of HIV by P-TEFb can be found in Refs. [45–47] and Fig. 3A. However, the precise structure of 7SK RNA as a scaffold has not been determined [48]. There are two published secondary structure models. The first comes from Wassarman and Steitz, who used RNA and
Fig. 3 The role of 7SK in the transcription regulation of HIV. (A) 7SK is critical for the activity of positive transcription elongation factor b (P-TEFb). 7SK sequesters P-TEFb into the catalytically inactive 7SK small nuclear ribonucleoprotein (snRNP), thereby inhibiting the cyclin-dependent kinase activity of P-TEFb. Otherwise, P-TEFb can be released from the 7SK snRNP where P-TEFb is inactivated by HEXIM1 and regulated by protein kinase C (PKC) or mitogen-activated protein kinase (MAPK) agonists. P-TEFb can be recruited to the HIV long terminal repeat sequence (LTR) by NF-κB, super elongation complex (SEC), Tat or bromodomain-containing Protein 4 (Brd4). Upon recruitment, P-TEFb phosphorylates Ser2/Ser5 and also phosphorylates Spt5 in DRB-sensitivity-inducing factor and negative elongation factor, thus releasing the arrested RNA polymerase II (RNAPII) for elongation. HIV transcription starts at the transcription start site (TSS) which located downstream of TATA box. The promoter contains a TATA box and 3 SP1 sites while the enhancer is NF-κB binding sites. PKC or MAPK agonists facilitate the translocation of NF-κB to the nucleus and improve the synthesis of P-TEFb. It's worth noting that the similarity between transactivation response (TAR) and the first stem loop in 7SK small nucleus non-coding RNA (snRNA) allows Tat and HEXIM1 to bind to both structures. Tat also competes with HEXIM1 for P-TEFb. Therefore, upon sufficient amounts of Tat, HIV transcription continues despite increased levels of 7SK snRNP. Brd4 can also relieve inhibition of P-TEFb by HEXIM1. 7SK is pseudouridine-acidified at U250 by DKC1-box H/ACA RNP (ribonucleoprotein), which is vital for the stability of 7SK snRNP. (B) 7SK with a standard 7SK motif and an extended 5' stem loop in the P-TEFb-containing snRNP is in an ‘open’ conformation allowing HEXIM1 to bind and inhibit P-TEFb. La-associated protein 7 (LARP7) is associated with the 3' loop while methyl phosphate capping enzyme (MEPCE) is located in the 5'end. Upon disassociation of 7SK SNP, HEXIM1 is also released, which leads to an altered 7SK motif structure that is stabilized by heterogeneous ribonucleoproteins (hnRNPs), thus resulting in a ‘closed’ conformation. MEPCE is inhibited in this conformation. A 7SK motif switch enables HEXIM1 binding and subsequent recruitment of P-TEFb into 7SK SNP again.
NRON to these proteins does not affect the transcription of the NFAT gene. In contrast, by isolating these proteins, NRON is likely to regulate the subcellular localization of NFAT through the interaction between NRON and nuclear factors, which has been confirmed by the increase in nuclear localization of NFAT after NRON inhibition. Therefore, the differential expression of NRON in the life cycle of HIV regulates the activity of NFAT by enabling or inhibiting its nuclear transport [58]. It has been found that the early viral assistant protein Nef negatively regulates the expression of NRON, while the late viral assistant protein Vpu has the opposite effect. Nef and Vpu regulate NFAT activation by regulating the expression of NRON, which ultimately affects the replication of HIV [59]. For a long time, the regulation of NFAT protein has played a vital role in regulating HIV transcription in T cells. Specifically, NFAT promotes the transcriptional activity of HIV by binding to the kβ motif in the LTR region near the viral NF-κβ binding site and can synergistically increase the transcription of HIV with NF-κβ [60,61] (Fig. 4). Another study reported that NRON could also directly associate Tat with ubiquitin/proteasome components (including CIKL4B (Cullin4B) and PSMD11) to promote Tat degradation, thus effectively inhibiting viral transcription and promoting HIV latency. In contrast, depletion of NRON, especially binding to histone deacetylase inhibitors, leads to viral production reactivation. These results suggest that the regulation of NRON may be a new target for the development of latency reversal agents [62].

LincRNA-p21/PANDA

As a retrovirus, HIV integrates its DNA into the host cell genome to form a provirus after entering the host cell. To achieve integration, the IN cleaves the host cell's genomic DNA to produce double-strand breaks. Two unique p53-dependent IncRNAs, long intergenic noncoding RNA-p21 (lincRNA-p21) and PANDA (p21-related noncoding RNA DNA damage activation), are derived from the gene encoding the critical cell cycle regulator cyclin dependent kinase inhibitor 1A (CDKN1A) (also known asp21) promoter, a canonical transcriptional target of p53 [63,64,67,75], and have been identified in two published studies by comparing differentially expressed IncRNAs after HIV infection. Since p53-binding proteins play a crucial role in the cell response to the breakage of double-stranded DNA in the genome, it is not surprising that p53-dependent IncRNAs are differentially expressed during viral infection [65,66]. Mechanistically, lincRNA-p21 binds to heterogeneous ribonucleoprotein K (hnRNK), which is thought to be necessary for p53-induced inhibition to be located in the promoter of the p53 suppressor gene. Without this interaction between IncRNAs and hnRNK, p53 cannot induce transcriptional suppression. The experimental phenomenon above indicates that lincRNA p21 plays an indispensable role in the response of p53 to genomic instability [67]. Meanwhile, to avoid the p53 apoptosis pathway, HIV sequesters hnRNK in the cytoplasm through the mitogen-activated protein kinase kinase 1 (MAP2K1)/extracellular signal-regulated protein kinase 2 (ERK2) pathway and locates Hur (highly conserved Elav-like proteins) in the nucleus. Hur is a ubiquitous RNA-binding protein (RBP) that influences cell proliferation, survival, carcinogenesis, and stress and immune responses [68].
Overall, the above method allows the transcription of survival genes. By binding to areas rich in AU, Hur keeps RNA transcripts stable in response to environmental pressures, thereby preventing degradation [68]. In response to environmental stress, the stabilizing effect of Hur on mRNA transcripts occurs in the cytoplasm of these cells [69–72]. On the other hand, the localization of Hur in the nucleus reduces the overall stability of lincRNA p21 because it recruits let-7 (one miRNA recruiting RNA-induced silencing complex (RISC) components)/Ago2 (argonaute 2, a RISC component that cleaves target mRNA) to the lincRNA p21 transcript [73]. In general, MAP2K1/ERK2 activation protects infected cells from p53-induced apoptosis through lincRNA-p21 degradation and hnRNP-K sequestration (Fig. 5). These results suggest that MAP2K1/ERK2 inhibitors can be treated as a new intervention strategy to treat HIV-infected macrophages [74].

PANDA is the second p53-dependent lncRNA with significant downregulation during HIV infection [8], similarly induced in a p53-dependent manner. The activation of lincRNA-p21 promotes apoptosis through gene inhibition, while PANDA inhibits apoptosis by binding to a subunit of nuclear transcription factor Y (NF-YA) and preventing it from coexisting in the promoter region of apoptotic genes (Fig. 5). NF-YA is a nuclear transcription factor that activates the p53-responsive promoter of Fas cell surface death receptor (FAS) upon DNA damage. The decrease in PANDA after HIV infection supports p53-induced apoptosis in chronically infected Jurkat T cells [75]. Because HIV induces double-strand breaks to integrate into the host genome, changes in the expression of these two lncRNAs (lincRNA-p21/PANDA) can be expected. Notably, an abundance of evidence has shown that HIV has evolved a mechanism to reduce the effect of p53 by directly targeting specific lncRNA pathways, and it can inhibit the apoptosis of host cells through this process. Upon detecting the survival of different types of immune cells, the crucial role of lincRNA-p21 in determining the fate of HIV-infected cells can be observed. Together, these findings demonstrate the significance of lncRNAs in disease progression.

**Lnc uc002yug2**

LncRNA uc002yug2, derived from the LINC01426 gene located on chromosome 21 [76], 2564 nt in length, is also involved in HIV replication and latency. This lncRNA is highly expressed in esophageal squamous cell carcinoma. LncRNA uc002yug2 enhances carcinogenesis by promoting the alternative splicing of runt-related transcription factor 1 (RUNX1) to increase the production of RUNX1a subtypes. RUNX1 is involved in HIV-1 latency and has been shown to repress HIV-1 replication in T cells by binding with the HIV-1 LTR [77]. Alternatively, uc002yug.2 was later proven to be present in complex regulatory networks controlling viral gene expression. Uc002yug.2 can potentially enhance HIV replication, LTR activity and the activation of latent HIV from HIV-infected cell lines and CD4+ T cells. Further investigation found that overexpression of uc002yug.2 could upregulate Tat protein expression. Simultaneously, the downregulation of uc002yug.2 mediated by short hairpin RNA led to a corresponding increase in the levels of RUNX1b and RUNX1c. In general, uc002yug.2 promotes HIV-1 LTR-driven transcription and latent HIV-1 reactivation through two mechanisms: 1. regulating the alternative splicing of pre-RUNX1 mRNA and downregulating the expression of the transcriptional inhibitors RUNX-1b and RUNX-1c; 2. upregulating the expression of Tat [78] (Fig. 6). These findings emphasize the value of uc002yug.2 as a potential HIV activator, thus indicating its role as a potential therapeutic target.
The newly inhibitory lncRNA AK130181 (also named LOC105747689), which is derived from the 3'UTR of the nucleic acid-binding protein 1 (NABP1) gene and highly expressed in CD4+ T lymphocytes latently infected with HIV, can inhibit LTR-driven HIV-1 gene transcription in an NF-kB-dependent manner in HIV-1 latency [66] (Fig. 7). Moreover,
viral production from HIV-1 latently infected Jurkat T cells and primary CD4+ T cells was significantly reactivated by silencing AK130181. Notably, inhibiting AK130181 in resting CD4+ T cells derived from HIV-1-infected individuals with highly active antiretroviral therapy results in increased viral reactivation upon T cell activation in vivo. This example provides a better understanding and new insights into lncRNAs that play a role in HIV-1 latency and suggests that it may be a potential therapeutic target for HIV-infected individuals by restricting AK130181 expression to activate HIV-1 latently infected cells [79].

Other lncRNAs in HIV infection

MALAT1
MALAT1 (metastatic-associated lung adenocarcinoma transcript 1) is an 8 kb, highly conserved nuclear-restricted ncRNA that is expressed from chromosome 11q13 [80] and can promote HIV-1 transcription. MALAT1 prevents the binding of the core component enhancer of Zest Homolog 2 (EZH2) to the promoter of HIV-1 LTR. EZH2 can result in viral transcription silencing and regulate the establishment and maintenance of HIV-1 latency by mediating histone H3 lysine 4 di-methylation (H3K27me3) on the LTR of the HIV-1 provirus [81]. Thus, MALAT1 eliminates PRC2 complex-mediated H3K27me3 and alleviates the epigenetic silencing of HIV-1 transcription [82].

NEAT1
NEAT1 (nuclear accessory dot assembly transcript 1) is a 4 kb, polyadenylated, unspliced and nuclear-restricted noncoding transcript with a mouse homolog that presents two small regions of high conservation and is derived from chromosome 11 [83]. NEAT1 is thought to be related to the innate immune response against viral infection. Some studies have proven that NEAT1 is essential for the substructural integrity of nuclear paraspeckles, and the nuclear paraspeckle body is regarded as a critical subcellular organelle for HIV replication [8].

HEAL
Another newly discovered lncRNA related to HIV replication and latency is HEAL (HIV-enhanced lncRNA). HEAL is derived from the chromosome 1 reverse strand and is conserved only in chimpanzees and rhesus monkeys [84]. Recent studies found that HEAL was upregulated following HIV infection and could promote HIV replication in monocyte-derived macrophages, microglia, and T lymphocytes. Additionally, HEAL binds to the RBP FUS to form the HEAL-FUS complex and promotes the replication of HIV by enhancing the recruitment of coactivator p300 (histone acetyltransferase) to the HIV promoter and stimulating the expression of cyclin-dependent kinase 2 [84].

SAF
Apart from CD4+ T lymphocytes, macrophages in tissue are also regarded as targets for HIV. This lncRNA SAF is derived from the opposite strand of Fas [85] and has the ability to regulate the apoptotic effect of caspases in macrophages. It has been suggested that SAF has the potential to be a therapeutic target for HIV reservoirs [86].

GAS5
A recent study found that the expression of GAS5 was downregulated during HIV infection. GAS5 is derived from the 5'-terminal oligopyrimidine (5’TOP) gene [87] and can act as a ceRNA to inhibit the expression of miR-873, thus weakening the replication of HIV. Together, these findings suggest that lncRNA GAS5 may be a potential target for developing new treatments for HIV/AIDS [88].

TAR-gag: Similar to 7SK and NRON, a new HIV lncRNA, TAR-gag, is derived from the gene located at two sites, either at the LTR or early gag regions [89], and can also induce the silencing and latency of genes in HIV transcription. The HIV transcription inhibitor F071613, a Tat-like peptide, can inhibit Tat binding to the CDK9/cycT1 complex and increase the synthesis of TAR-gag. Importantly, TAR-gag is likely to act as an “RNA machine” and binds with inhibitory transcriptional
proteins to form an RNA-protein complex regulating HIV gene expression [90].

HIV-encoded antisense IncRNA: Viruses can utilize either host-encoded IncRNAs or virus-encoded IncRNAs to promote infection and persistence. An antisense IncRNA encoded by HIV has been reported to regulate the expression of viral genes. This viral IncRNA is transcribed in the opposite direction of overlapping protein-coding genes and localized to the 5’ LTR. In the process of IncRNA-induced epigenetic gene silencing, the viral IncRNA was found to be located at the 5’ end of the transcription factor and usurped the components of the endogenous cellular pathway, thereby inhibiting the expression of viral genes. Moreover, inhibition of this viral IncRNA with small single-stranded antisense RNA can activate viral gene expression. This antisense IncRNA encoded by HIV is considered an epigenetic brake for regulating virus transcription by altering the epigenetic pattern of viral promoters [9].

LINC00173

Researchers analyzed two independently generated RNA-SEQ datasets to determine the differentially expressed IncRNAs following HIV infection. They selected LINC00173 for further study. LINC00173 is a large intergenic noncoding RNA (lncRNA) derived from chromosome 12 [91], and its expression has been found to be increased during infection. However, using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas9) to knock out LINC00173 had no significant effects on HIV replication. Interestingly, LINC00173-deficient Jurkat cells expressed more specific cytokines on average than control cells. These findings suggest that LINC00173 is likely to regulate cytokine levels in T cells during the immune response [91].

The clinical significance and challenges of IncRNAs in HIV infection

Undoubtedly, IncRNAs are likely to be applied in the clinical environment due to their association with HIV infection both in vitro and in vivo. Although there are relatively few studies on the therapeutic role of IncRNAs in HIV infection in vivo, the crucial role of IncRNAs in cancer has been well elucidated [92]. IncRNAs have now been used as candidate biomarkers for diseases. High stability, tissue specificity, and ubiquity in various body fluids make IncRNAs suitable as clinical indicators [88]. In respect to HIV, a study on GAS5 proposed the ceRNA mechanism in the immunopathogenic cross-talk of AIDS and revealed that GAS5 might be a good target for increasing antiviral drug efficacy [88]. Jin et al. found that the expression of NEAT1 in plasma is related to CD4+ T cell count, suggesting that NEAT1 may be a promising biomarker of disease progression [93].

Because IncRNAs are similar to miRNAs in their transcriptional processes and have structural similarities, their production is considered to be regulated similarly to that of protein-coding genes. The mechanisms include DNA and histone modifications, RNA splicing and transcription factor binding, but few of these areas in IncRNA biology have been explored. One example is that the expression of IncRNA TERRA could be suppressed by DNMT1 and DNMT3b-mediated methylation of Cpg dinucleotides in the human subtelomeric region. H3K9 histone methyltransferase SUV39H1 and HP1α protein, which can bind H3K9me3, also restrain TERRA transcription [94]. IncRNA stability can be affected by miRNA. For example, EVN-mir-BART6-3p can inhibit the migration and metabolism of tumor cells by targeting and downregulating IncRNA-LOC553103 in EVB-associated cancer tissues and cells [95]. In addition, a study indicated that miRNAs encoded by KSHV could reduce host IncRNAs in a miRNA-dependent manner [96]. Another study showed a reciprocally negative regulation between miR-21 and GAS5. Mir-21 represses GAS5 by targeting a sequence encoded by its exon 4, and GAS5 also inhibits mir-21 expression in turn [97].

IncRNAs can be interfered with by various biological tools, including RNA interference-mediated gene silencing, antisense oligonucleotide (ASO), CRISPR/Cas9 systems, small-molecule compounds and plant-derived natural compounds. Specifically, siRNAs, which are short (19–30 nt) double-stranded RNAs, recruit the RISC to cause IncRNA degradation complementary to the RNA sequence. For example, siRNA was applied to target different sites within IncRNA CASC9, and two sites, CASC9-2 and CASC9-3, were defined to have the highest knockout efficiency [98]. ASOs, which are a type of short single-stranded oligonucleotide that have a 15- to 25-nucleotide sequence, modulate IncRNA function through degradation of IncRNA transcripts. RNase H1 identifies the DNA:RNA heteroduplex and catalyzes the cleavage of RNA molecules after ASOs bind to target complementary RNAs. For example, a treatment trial for Angelman syndrome used ASOS targeting IncRNA Ube3a-ATS to achieve the specific reduction of Ube3a-ATS in neurons and continuous desilencing paternal Ube3a alleles in vitro and in vivo [99]. The CRISPR/Cas9 system, which is an immune defense system for prokaryotes, contains a single guide RNA (sgRNA) and a Cas9 enzyme. SgRNA directs the Cas9 nuclease to concrete sites in the genome by complementary base pairing, and Cas9 cleaves the DNA sequence adjusted to the protoscaler-adjacent motif. CRISPR/Cas9 has been favorably exploited to target IncRNAs owing to its excellent efficiency, permanence, accuracy, and ease of programming [100,101]. For example, a mouse model of IncRNA Norad, which is activated by DNA damage and deletion, was established using the CRISPR/Cas9 system, and Norad-deficient mice displayed fine similarities in the multisystem degenerative phenotype of premature aging, causing severe mitochondrial dysfunction and genomic instability [102].

The cellular location of IncRNAs should be premeditated when selecting siRNA, ASOs or CRISPR/Cas9 for IncRNA targeting in preclinical models. SiRNAs can more effectively inhibit cytoplasmic IncRNAs, ASOs can more effectively inhibit nuclear IncRNAs, and CRISPR/Cas9 can be employed for IncRNA knockout regardless of cellular location but has a relatively narrow IncRNA spectrum. Therefore, combining these methods together makes it easier to realize valid interference for IncRNAs with dual or unclear cellular localization. In addition to nucleic acid technology, small-molecule compounds have been confirmed to be effective entities because they disrupt IncRNA dimensional structure or IncRNA–protein
technology to quantify lncRNA–protein interactions and determine a small molecule, ellipticine, that could inhibit the interaction of HOTAIR–EZH2 and BDNF-AS–EZH2. This example demonstrated that the application of high-throughput screening to evaluate lncRNA–protein interactions and target lncRNAs with small molecules was promising. It is unclear whether small molecules will precede nucleic acid technology for the evolution of lncRNA-based drugs, but small-molecule inhibitors have the advantages of easier administration modes and lower cost compared with nucleic acid drugs. More importantly, small molecules target lncRNAs using a structure–designated method rather than a sequence–complementary method and may coordinate lncRNA functions without changing their expression. This coordination cannot be realized by siRNAs, ASOs, and CRISPR, since small molecules can disturb the binding or interaction between lncRNAs and other biomolecules. Studies have shown that plant-derived natural compounds possess reliable regulation of lncRNAs. For example, curcumin, which is a natural molecule with anticancer functions, can downregulate H19 and facilitate the expression of p53 in a concentration- and time-dependent manner, indicating the capacity of phytochemicals to modulate lncRNAs. However, phytochemicals lack accurate targets and mechanisms, which is a shortcoming.

Until now, people have considered lncRNAs to be ‘byproducts’ of normal transcription, but actually, an increasing number of lncRNAs have displayed the potential for independent transcription. For example, lincRNA-p21, which are defined as autonomously transcribed noncoding RNAs longer than 200 nucleotides that do not overlap annotated coding genes, account for more than half of lncRNA transcripts in humans. Therefore, using nucleic acid tools, including siRNAs, ASOs and the CRISPR/Cas9 system, to target lincRNAs will not affect the transcription of coding genes. Although many lncRNAs can be transcribed by protein-coding genes, the majority of lncRNAs contain intronic regions. Therefore, nucleic acid tools might not affect the transcription of the genes from which these lncRNAs are produced by targeting the intronic region. In addition, small molecules disturb the binding or interaction between lncRNAs and other biomolecules and might not affect the transcription of the genes. For plant-derived natural compounds, more investigations are needed to determine the targeting specificity and mechanisms.

Although lncRNAs have shown therapeutic potential, developing a reliable delivery system remains a significant challenge. The system must have adequate stability, specificity, cell permeability, and low immunogenicity. Considering the substantial potential therapeutic value of lncRNAs, we should be optimistic about applying lncRNAs in the treatment of HIV. However, it is notable that reports on the role of lncRNAs in HIV infection are almost all based on in vitro analysis using HIV-infected cell line models, while only a few are based on primary cell models. Obviously, these results require further exploration and verification in animal models to fully understand the role of lncRNAs in HIV infection. Indeed, numerous unknowns and technical limitations in the research of lncRNAs still render their corresponding application challenging. In addition, the safety, cost and ethical issues of lncRNA-mediated therapy should be confirmed in nonhuman primates and clinical trials. Notably, eradicating HIV latent reservoirs remains a significant challenge to the thorough cure of HIV infection. Scientists around the world are dedicated to developing novel methods to treat latent HIV, which can currently be divided into the following categories: gene therapy, silencing HIV, “shocking” the latent period of HIV, and “killing” infected cells. Although none of these strategies can eradicate latent HIV alone, the combination of these methods may have better results. In the future, with more advanced techniques, more fascinating features and functions of lncRNAs will be undoubtedly found, which in turn may be beneficial to the treatment of different human diseases.

Declaration of competing interest

The authors declare that they have no competing interests.

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