Review Article

Roles of lncRNAs in the transcription regulation of HIV-1

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ARTICLE INFO

ABSTRACT

Long noncoding RNAs (LncRNAs) is a class of RNA molecules that are more than 200bp but cannot be translated into proteins. More and more studies have proved that lncRNA plays a crucial role in various biological functions and disease processes, including virus infection. It's worth noting that studies have also shown that lncRNAs 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 lncRNA-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 lncRNAs 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 lncRNAs 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 lncRNAs might help develop non-traditional antiviral therapy strategies.

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 lncRNAs 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].
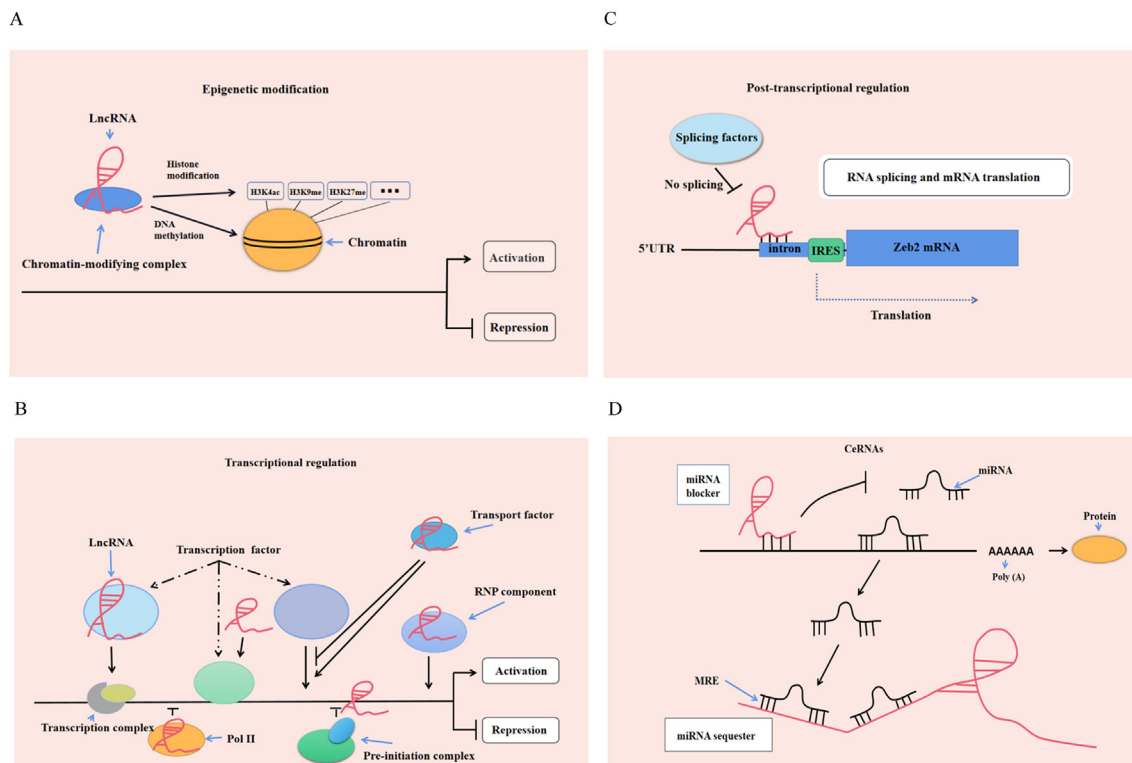

Fig. 1 **Four mechanisms of lncRNA in the regulation of gene expression.** (A) Epigenetic modification: LncRNA can bind to one or more chromatin modification complexes, recruit the complexes to specific genome loci, where they acetylate or methylate lysine residues of histone H3 as well as methylate 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.
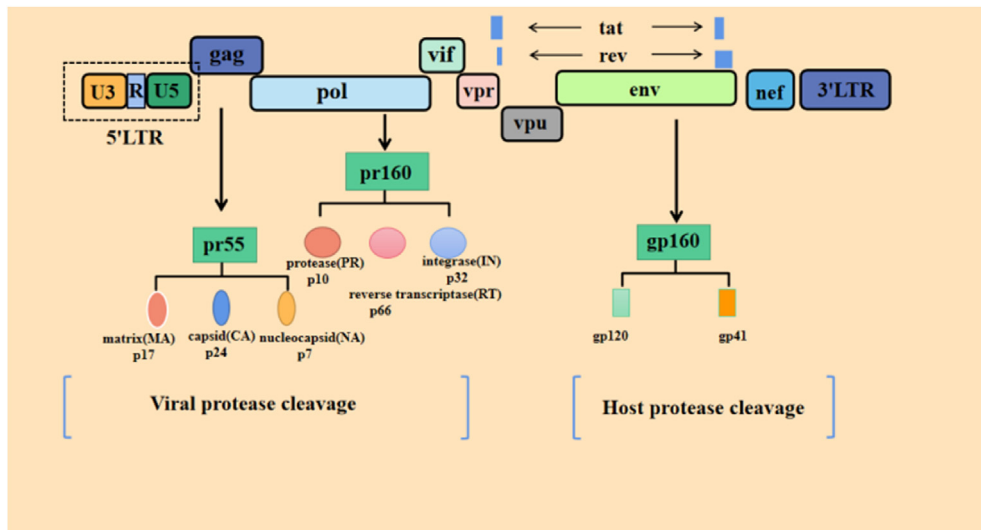
Fig. 2 **The arrangement of HIV genome.** Nine genes are encoded by the HIV genome. Group-specific antigen (Gag), Pol, and Env genes are further processed into pr55, pr160 and gp160. pr55 are finally cleaved into p17, p24 and p7 by the viral protease (PR). pr160 are cleaved into p10, p32 and p66 also by the viral PR. Gp160 is cleaved into gp120 and gp41 by the host PR.

Epigenetic modification

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 *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 lncRNA 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 lncRNA 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, lncRNA THRIL and lncRNA-COX2 regulate the transcription of TNF-α and chemokine (CeC 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 lncRNAs 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].

The structure and protein functions of HIV

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 transcripts (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 cytoproteases 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 gp41 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 multispliced transcript synthesized early in the life cycle of the virus. It binds to the transactivation response (TAR) element in the R domain of the 5′ 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 5′ 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 9.8 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-kB 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 phosphate 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-mono methyl cap to protect the 5′ 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 (trans-activating 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
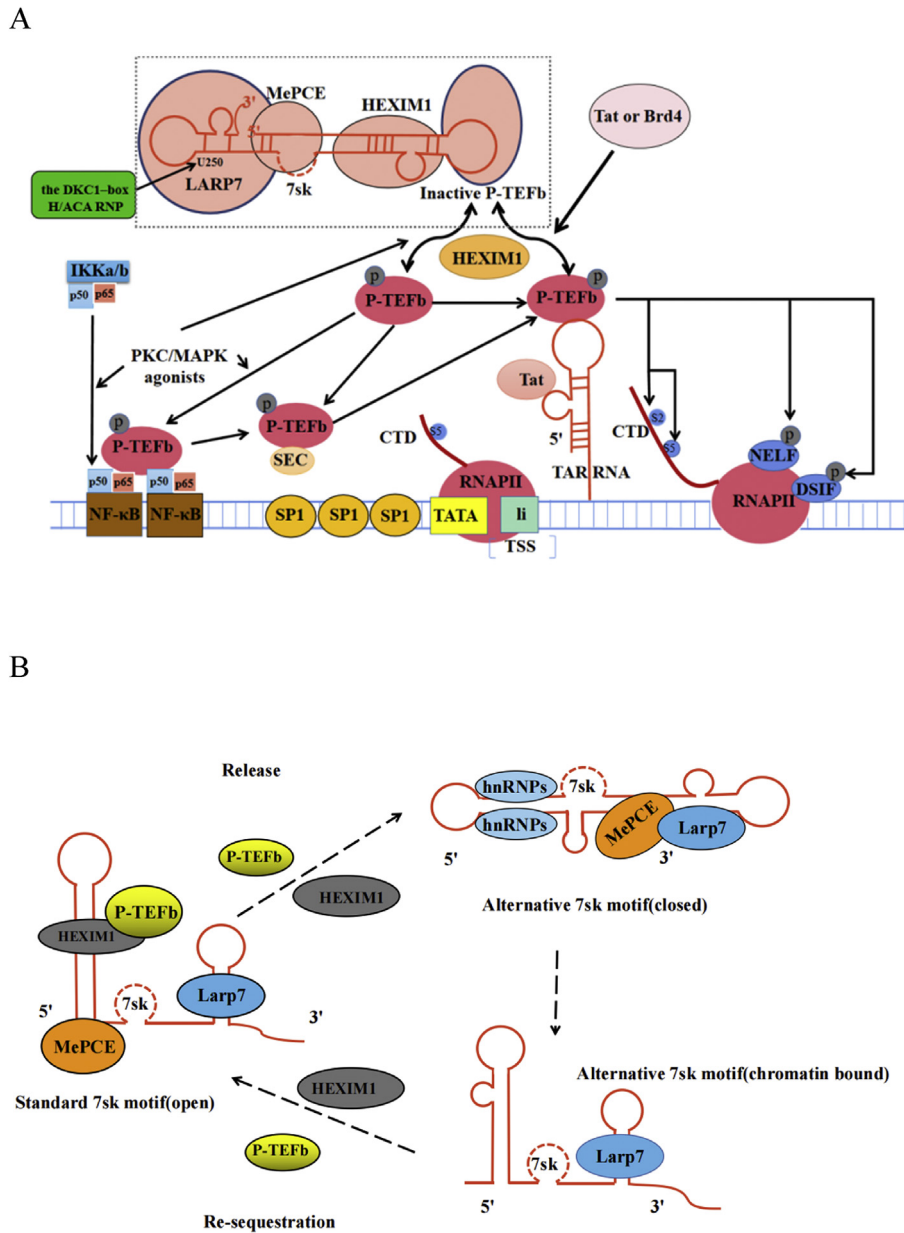
Fig. 3 **The role of 7SK in the transcription regulation of HIV.** (A) 7SK is critical for the activity of positive transcription elongation factor b (P-TEFb). 7SK sequesters P-TEFb into the catalytically inactive 7SK small nuclear ribonucleoprotein (snRNP), thereby inhibiting the cyclin-dependent kinase 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 disaccociation 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.

RNP to predict the structure [49]. The second is from Marz et al. who added evolutionary conservation to the model [50]. The 5′ end of 7SK predicted by the two models is clearly different, and these two structures have been adopted to some extent for research. 7SK experienced magnesium-dependent conformational changes *in vitro*, resulting in the structural rearrangement of 7SK induced by magnesium. This phenomenon further confirms that 7SK has two different conformations, which may be close in energy. One may be beneficial to low magnesium, and the other may be beneficial to high magnesium [48,52]. Furthermore, the 7SK motif switch promotes changes in the 7SK motif. Here, '7SK motif' is a short helix formed by pairing GAUC2 (nt 42—45) and GAUC3 (nt 64—67), which is flanked by unpaired uridine, and the concept of '7SK motif switch' is introduced in Fig. 3B; it can play a crucial role in regulating the activity of P-TEFb by combining with HEXIM1 [48,51—53]. Upon P-TEFb and GAUC3 (nt 64—67) pairing to form 7SK motifs, the binding of P-TEFb and HEXIM1 [48,51—53] may play a key role in regulating the activity of P-TEFb. When P-TEFb is released, HEXIM1 binding decreases, and hnRNP (heterogeneous ribonucleoprotein) stabilizes the alternative 7SK motif. The storage of P-TEFb may occur through the 7SK binding of chromatin-related LARP7 containing alternative 7SK motifs. The recombination of HEXIM1 and P-TEFb triggers the 7SK motif switch, which converts 7SK back to open snRNP [48] (Fig. 3B). There are new insights into the structure of 7SK RNA and its effect on HIV transcription, and the remaining problems are mainly related to how P-TEFb is reisolated. Subsequently, whether and how P-TEFb is removed from snRNP and transferred to genes deserves further study.

7SK snRNA isolates P-TEFb into catalytically inactivated 7SK snRNP, but little is known about how 7SK RNA is regulated to perform this function [54]. Genome-wide studies suggest that 7SK RNA may undergo a posttranscriptional modification called pseudouridine acidification, altering its structure and function [55,56]. Pseudouracil plays an essential role in the stability of 7SK snRNP. 7SK is pseudouridine-acidified by DKC1-Box H/ACA RNP, the primary cellular pseudouracil synthetase mechanism at U250. When this modification is lost due to a mutation in or near the U250 or because of the depletion of the catalytic component DKC1 of Box H/ACA RNP, this condition destroys the 7SK snRNP and releases the P-TEFb, promoting the reversal of viral latency by enhancing the binding of super elongation complex to Tat and HIV-1 LTR [54] (Fig. 3A).

NRON

NRON (noncoding repressor of T cells, T cell nuclear factor noncoding inhibitor) is derived from one gene that is composed of three exons and can be alternatively spliced to yield transcripts ranging in size from 0.8 to 3.7 kb [58]. Studies have shown that NRON can regulate HIV transcription at different stages of the HIV life cycle. NRON regulates the expression of nuclear factor of activated T cells (NFAT) protein to inactive T cells by binding to calmodulin-binding protein (IQGAP1), nuclear transport factor (KPNB1), phosphatase (PPP2R1A), and proteasome (PSMD11) [58]. This NFAT protein is an extremely sensitive transcription factor that reacts to local changes in calcium signals and is necessary for the T cell receptor-mediated immune response [57]. Interestingly, the binding of

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 kB motif in the LTR region near the viral NF-kB binding site and can synergistically increase the transcription of HIV with NF-kB [60,61] (Fig. 4). Another study reported that NRON could also directly associate Tat with ubiquitin/proteasome components (including CUL4B (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 lncRNAs, 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 lncRNAs 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 lncRNAs are differentially expressed during viral infection [65,66]. Mechanistically, lincRNA-p21 binds to heterogeneous ribonucleoprotein K (hnRNP-K), 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 lncRNAs and hnRNP-K, 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 hnRNP-K 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].
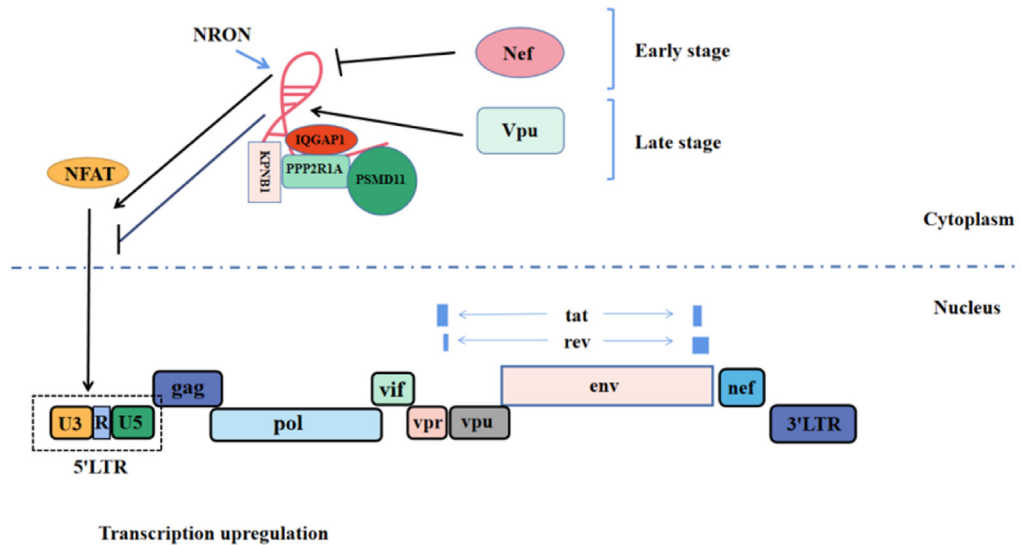
Fig. 4 NRON indirectly regulates HIV transcription. NRON can regulate HIV transcription by inhibiting the nuclear transport of nuclear factor of activated T cells (NFAT) through the assembly of IQGAP1, KPNB1, PPP2R1A and PSMD11. In the early stage of virus infection, negative factor (Nef) inhibits NRON expression resulting in accumulation of NFAT in the nucleus and promoting virus transcription. On the contrary, in the late stage of virus infection, viral protein U (vpu) can induce the expression of NRON, hence inhibiting the relocation of NFAT and virus transcription.

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 uc002yug.2, 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 uc002yug.2 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.
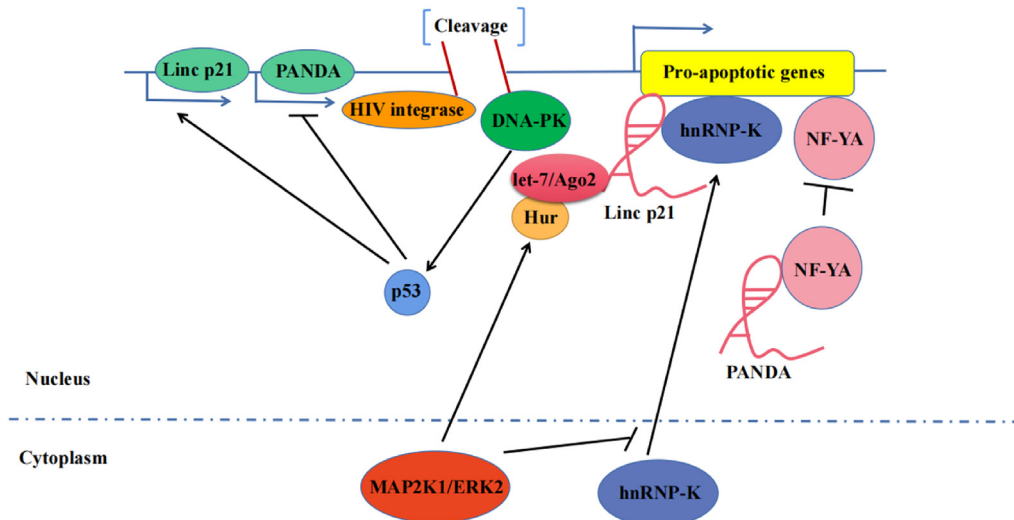
Fig. 5 **The role of p53-dependent long intergenic noncoding RNA-p21 (lincRNA-p21)/PANDA in HIV transcription regulation and infection.** The HIV integrase cleaves the host cell DNA before the viral genome integration, resulting in a double-strand break and DNA-PK activation. DNA-PK phosphorylates p53 to produce cascade signal amplification and eventually leads to apoptosis. LincRNA-p21 induced by p53 can bind to hnRNP-K and enable the expression of pro-apoptotic genes. To prevent this pathway, the initiation of the mitogen-activated protein kinase kinase 1 (MAP2K1)/extracellular signal-regulated protein kinase 2 (ERK2) pathway was induced by HIV, thus causing hnRNP-K and Hur to be sequestered in the cytoplasm and nucleus respectively. Hur subsequently can recruits let-7/Ago2 to the lincRNA p21. Thus, MAP2K1/ERK2 activation protects HIV-infected cells against p53-induced apoptosis. On the contrary, PANDA is able to inhibit apoptosis through binding to a subunit of nuclear transcription factor Y (NF-YA) and preventing its re-localization at promoter regions of apoptotic genes.
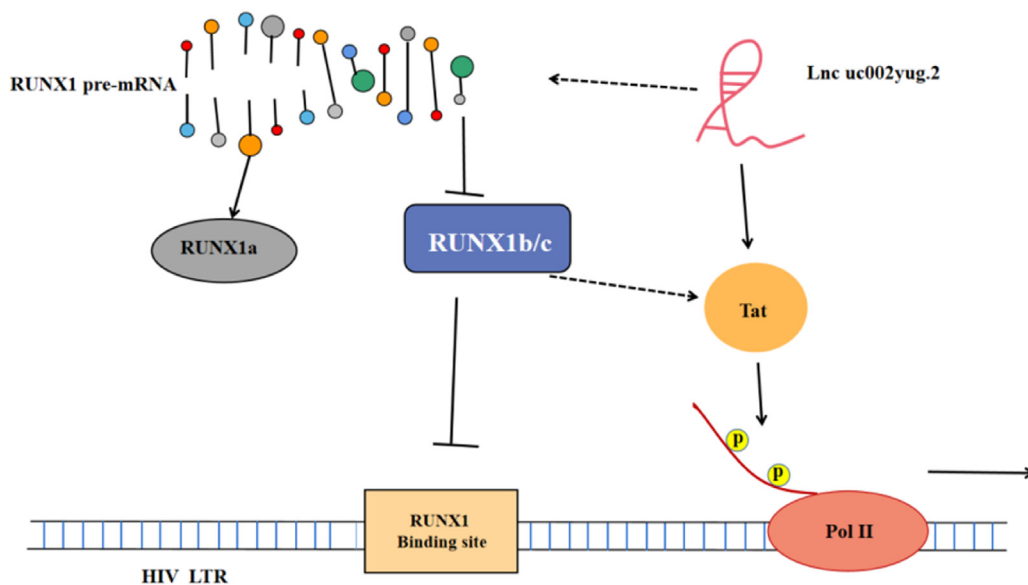

Fig. 6 **The function of Lnc uc002yug.2 in HIV reactivation.** UC002yug.2 may promote the replication and reactivation of HIV-1 through two mechanisms: downregulation of RUNX1b and-1c and upregulation of Tat. RUNX1b and-1c are downregulated due to the alternative splicing of pre-RUNX1-mRNA upon uc002yug.2 increasement, thus reducing the inhibition of RUNX1b and-1c on HIV-LTR activity. Meanwhile, Tat protein is upregulated and transactivated to promote HIV-LTR activity. The dotted arrow represents the hypothetical potential path.

lncRNA AK130181

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,
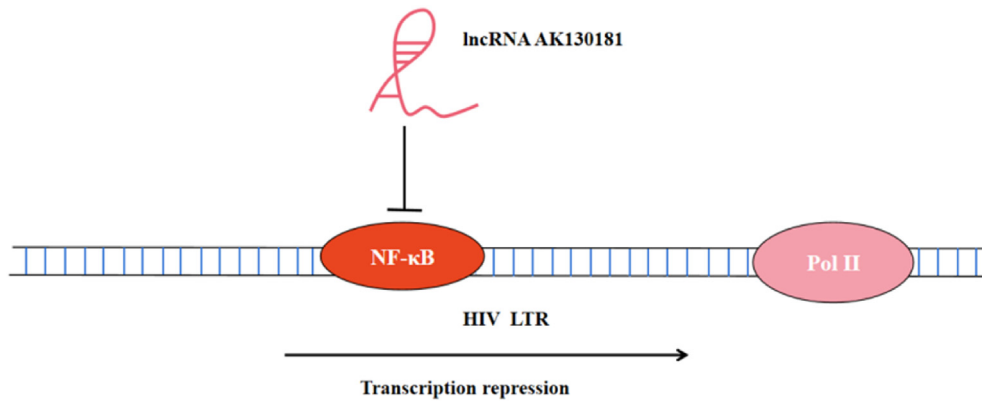
Fig. 7 **The role of Lnc AK130181 in HIV transcription.** AK130181 inhibits LTR-driven HIV-1 gene transcription in a NF-κB-dependent manner and is involved in HIV-1 latency.

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 Zust 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 lncRNA: Viruses can utilize either host-encoded lncRNAs or virus-encoded lncRNAs to promote infection and persistence. An antisense lncRNA encoded by HIV has been reported to regulate the expression of viral genes. This viral lncRNA is transcribed in the opposite direction of overlapping protein-coding genes and localized to the 5' LTR. In the process of lncRNA-induced epigenetic gene silencing, the viral lncRNA 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 lncRNA with small single-stranded antisense RNA can activate viral gene expression. This antisense lncRNA 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 lncRNAs following HIV infection. They selected LINC00173 for further study. LINC00173 is a large intergenic noncoding RNA (lincRNA) 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 lncRNAs in HIV infection

Undoubtedly, lncRNAs 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 lncRNAs in HIV infection *in vivo*, the crucial role of lncRNAs in cancer has been well elucidated [92]. LncRNAs have now been used as candidate biomarkers for diseases. High stability, tissue specificity, and ubiquity in various body fluids make lncRNAs 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 lncRNAs are similar to mRNAs 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 lncRNA biology have been explored. One example is that the expression of lncRNA 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]. lncRNA stability can be affected by miRNA. For example, EBV-miR-BART6-3p can inhibit the migration and metabolism of tumor cells by targeting and downregulating lncRNA-LOC553103 in EBV-associated cancer tissues and cells [95]. In addition, a study indicated that miRNAs encoded by KSHV could reduce host lncRNAs 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].

LncRNAs 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 lncRNA degradation complementary to the RNA sequence. For example, siRNA was applied to target different sites within lncRNA 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 lncRNA function through degradation of lncRNA 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 lncRNA 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 protospacer-adjacent motif. CRISPR/Cas9 has been favorably exploited to target lncRNAs owing to its excellent efficiency, permanence, accuracy, and ease of programming [100,101]. For example, a mouse model of lncRNA 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 lncRNAs should be premeditated when selecting siRNA, ASOs or CRISPR/Cas9 for lncRNA targeting in preclinical models. SiRNAs can more effectively inhibit cytoplasmic lncRNAs, ASOs can more effectively inhibit nuclear lncRNAs, and CRISPR/Cas9 can be employed for lncRNA knockout regardless of cellular location but has a relatively narrow lncRNA spectrum. Therefore, combining these methods together makes it easier to realize valid interference for lncRNAs 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 lncRNA dimensional structure or lncRNA–protein

interactions. Pedram Fatemi et al. exploited ALPHA screening 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 [103]. 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 [104−106]. 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 [107], 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 [108]. 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 [109−111]. 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 [112,113]. 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.

Acknowledgment

REFERENCES

[1] Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007;316:1484−8.

[2] Ratner L, Haseltine W, Patarca R, Livak KJ, Starcich B, Josephs SF, et al. Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 1985;313:277−84.

[3] Sanchez-Pescador R, Power MD, Barr PJ, Steimer KS, Stempien MM, Brown-Shimer SL, et al. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). Science 1985;227:484−92.

[4] Wain-Hobson S, Sonigo P, Danos O, Cole S, Alizon M. Nucleotide sequence of the AIDS virus. LAV. Cell 1985;40:9−17.

[5] Garitano-Trojaola A, Agirre X, Prósper F, Fortes P. Long non-coding RNAs in haematological malignancies. Int J Mol Sci 2013;14:15386−422.

[6] Ponjavic J, Ponting CP, Lunter G. Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. Genome Res 2007;17:556−65.

[7] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010;464:1071−6.

[8] Zhang Q, Chen CY, Yedavalli VS, Jeang KT. NEAT1 long noncoding RNA and paraspeckle bodies modulate HIV-1 posttranscriptional expression. mBio 2013;4. e00596-12.

[9] Saayman S, Ackley A, Turner AW, Famiglietti M, Bosque A, Clemson M, et al. An HIV-Encoded antisense long noncoding rna epigenetically regulates viral transcription. Mol Ther 2014;22:1164−75.

[10] Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. Mol Cell 2011;43:904−14.

[11] Bonasio R, Shiekhattar R. Regulation of transcription by long noncoding RNAs. Annu Rev Genet 2014;48:433–55.

[12] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010;329:689–93.

[13] Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science 2008;322:1717–20.

[14] Rapicavoli NA, Qu K, Zhang J, Mikhail M, Laberge RM, Chang HY. A mammalian pseudogene lncRNA at the interface of inflammation and anti-inflammatory therapeutics. Elife 2013;2:e00762.

[15] Carpenter S, Aiello D, Atianand MK, Ricci EP, Gandhi P, Hall LL, et al. A long noncoding RNA mediates both activation and repression of immune response genes. Science 2013;341:789–92.

[16] Li Z, Chao TC, Chang KY, Lin N, Patil VS, Shimizu C, et al. The long noncoding RNA THRIL regulates TNFα expression through its interaction with hnRNPL. Proc Natl Acad Sci U S A 2014;111:1002–7.

[17] Beltran M, Puig I, Peña C, García JM, Álvarez AB, Peña R, et al. A natural antisense transcript regulates Zeb2/Sip1 gene expression during Snail1-induced epithelial-mesenchymal transition. Genes Dev 2008;22:756–69.

[18] Sen R, Ghosal S, Das S, Balti S, Chakrabarti J. Competing endogenous RNA: the key to posttranscriptional regulation. Sci World J 2014;2014:896206.

[19] Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell 2011;147:358–69.

[20] Barré-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220:868–71.

[21] Gallo RC, Sarin PS, Gelmann EP, Robert-Guroff M, Richardson E, Kalyanaraman VS, et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 1983;220:865–7.

[22] Ganser-Pornillos BK, Yeager M, Sundquist WI. The structural biology of HIV assembly. Curr Opin Struct Biol 2008;18:203–17.

[23] Hill M, Tachedjian G, Mak J. The packaging and maturation of the HIV-1 pol proteins. Curr HIV Res 2005;3:73–85.

[24] Moulard M, Decroly E. Maturation of HIV envelope glycoprotein precursors by cellular endoproteases. Biochim Biophys Acta 2000;1469:121–32.

[25] Salzwedel K, Smith ED, Dey B, Berger EA. Sequential CD4-coreceptor interactions in human immunodeficiency virus type 1 env function: soluble CD4 activates env for coreceptor-dependent fusion and reveals blocking activities of antibodies against cryptic conserved epitopes on gp120. J Virol 2000;74:326–33.

[26] Campbell EM, Hope TJ. Live cell imaging of the HIV-1 life cycle. Trends Microbiol 2008;16:580–7.

[27] Dvorin JD, Malim MH. Intracellular trafficking of HIV-1 cores: journey to the center of the cell. Curr Top Microbiol Immunol 2003;281:179–208.

[28] Eilebrecht S, Benecke BJ, Benecke AG. Latent HIV-1 TAR regulates 7SK-responsive P-TEFb target genes and targets cellular immune responses in the absence of tat. Genomics Proteomics Bioinformatics 2017;15:313–23.

[29] Pollard VW, Malim MH. The HIV-1 Rev protein. Annu Rev Microbiol 1998;52:491–532.

[30] Anderson JL, Hope TJ. HIV accessory proteins and surviving the host cell. Curr HIV/AIDS Rep 2004;1:47–53.

[31] Emerman M, Malim MH. HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. Science 1998;280:1880–4.

[32] Patarca R, Heath C, Goldenberg GJ, Rosen CA, Sodroski JG, Haseltine WA, et al. Transcription directed by the HIV long terminal repeat in vitro. AIDS Res Hum Retroviruses 1987;3:41–55.

[33] Roebuck KA, Saifuddin M. Regulation of HIV-1 transcription. Gene Expr 1999;8:67–84.

[34] Chiu YL, Cao H, Jacque JM, Stevenson M, Rana TM. Inhibition of human immunodeficiency virus type 1 replication by RNA interference directed against human transcription elongation factor P-TEFb (CDK9/CyclinT1). J Virol 2004;78:2517–29.

[35] Nguyen DG, Wolff KC, Yin H, Caldwell JS, Kuhen KL. "UnPAKing" human immunodeficiency virus (HIV) replication: using small interfering RNA screening to identify novel cofactors and elucidate the role of group I PAKs in HIV infection. J Virol 2006;80:130–7.

[36] Jacque JM, Stevenson M. The inner-nuclear-envelope protein emerin regulates HIV-1 infectivity. Nature 2006;441:641–5.

[37] Llano M, Saenz DT, Meehan A, Wongthida P, Peretz M, Walker WH, et al. An essential role for LEDGF/p75 in HIV integration. Science (80-) 2006;314:461–4.

[38] Vollenweider F, Benjannet S, Decroly E, Savaria D, Lazure C, Thomas G, et al. Comparative cellular processing of the human immunodeficiency virus (HIV-1) envelope glycoprotein gp160 by the mammalian subtilisin/kexin-like convertases. Biochem J 1996;314:521–32.

[39] Trypsteen W, Mohammadi P, Van Hecke C, Mestdagh P, Lefever S, Saeys Y, et al. Differential expression of lncRNAs during the HIV replication cycle: an underestimated layer in the HIV-host interplay. Sci Rep 2016;6:36111.

[40] Murphy S, Tripodi M, Melli M. A sequence upstream from the coding region is required for the transcription of the 7SK RNA genes. Nucleic Acids Res 1986;14:9243–60.

[41] Abasi M, Bazi Z, Mohammadi-Yeganeh S, Soleimani M, Haghpanah V, Zargami N, et al. 7SK small nuclear RNA transcription level down-regulates in human tumors and stem cells. Med Oncol 2016;33:128.

[42] Gurney T Jr, Eliceiri GL. Intracellular distribution of low molecular weight RNA species in hela cells. J Cell Biol 1980;87:398–403.

[43] Peterlin BM, Brogie JE, Price DH. 7SK snRNA: a noncoding RNA that plays a major role in regulating eukaryotic transcription. Wiley Interdiscip Rev RNA 2012;3:92–103.

[44] Yang Z, Zhu Q, Luo K, Zhou Q. The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. Nature 2001;414:317–22.

[45] Lu H, Li Z, Zhang W, Schulze-Gahmen U, Xue Y, Zhou Q. Gene target specificity of the Super Elongation Complex (SEC) family: how HIV-1 Tat employs selected SEC members to activate viral transcription. Nucleic Acids Res 2015;43:5868–79.

[46] Zhu J, Gaiha GD, John SP, Pertel T, Chin CR, Gao G, et al. Reactivation of latent HIV-1 by inhibition of BRD4. Cell Rep 2012;2:807–16.

[47] Itzen F, Greifenberg AK, Bösken CA, Geyer M. Brd4 activates P-TEFb for RNA polymerase II CTD phosphorylation. Nucleic Acids Res 2014;42:7577–90.

[48] Brogie JE, Price DH. Reconstitution of a functional 7SK snRNP. Nucleic Acids Res 2017;45:6864–80.

[49] Wassarman DA, Steitz JA. Structural analyses of the 7SK ribonucleoprotein (RNP), the most abundant human small RNP of unknown function. Mol Cell Biol 1991;11:3432–45.

[50] Marz M, Donath A, Verstraete N, Nguyen VT, Stadler PF, Bensaude O. Evolution of 7SK RNA and its protein partners in metazoa. Mol Biol Evol 2009;26:2821–30.

[51] Krueger BJ, Varzavand K, Cooper JJ, Price DH. The mechanism of release of P-TEFb and HEXIM1 from the 7SK snRNP by viral and cellular activators includes a conformational change in 7SK. PLoS One 2010;5:e12335.

[52] Lebars I, Martinez-Zapien D, Durand A, Coutant J, Kieffer B, Dock-Bregeon AC. HEXIM1 targets a repeated GAUC motif in the riboregulator of transcription 7SK and promotes base pair rearrangements. Nucleic Acids Res 2010;38:7749–63.

[53] Martinez-Zapien D, Legrand P, McEwen AG, Proux F, Cragnolini T, Pasquali S, et al. The crystal structure of the 5' functional domain of the transcription riboregulator 7SK. Nucleic Acids Res 2017;45:3568–79.

[54] Zhao Y, Karijolich J, Glaunsinger B, Zhou Q. Pseudouridylation of 7SK snRNA promotes 7SK snRNP formation to suppress HIV- 1 transcription and escape from latency. EMBO Rep 2016;17:1441–51.

[55] Carlile TM, Rojas-Duran MF, Zinshteyn B, Shin H, Bartoli KM, Gilbert WV. Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature 2014;515:143–6.

[56] Schwartz S, Bernstein DA, Mumbach MR, Jovanovic M, Herbst RH, León-Ricardo BX, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell 2014;159:148–62.

[57] Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. Genes Dev 2003;17:2205–32.

[58] Willingham AT, Orth AP, Batalov S, Peters EC, Wen BG, Aza-Blanc P, et al. A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. Science 2005;309:1570–3.

[59] Imam H, Bano AS, Patel P, Holla P, Jameel S. The lncRNA NRON modulates HIV-1 replication in a NFAT-dependent manner and is differentially regulated by early and late viral proteins. Sci Rep 2015;5:8639.

[60] Kinoshita S, Su L, Amano M, Timmerman LA, Kaneshima H, Nolan GP. The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells. Immunity 1997;6:235–44.

[61] Cron RQ, Bartz SR, Clausell A, Bort SJ, Klebanoff SJ, Lewis DB. NFAT1 enhances HIV-1 gene expression in primary human CD4 T cells. Clin Immunol 2000;94:179–91.

[62] Li J, Chen C, Ma X, Geng G, Liu B, Zhang Y, et al. Long noncoding RNA NRON contributes to HIV-1 latency by specifically inducing tat protein degradation. Nat Commun 2016;7:11730.

[63] Chen S, Liang H, Yang H, Zhou K, Xu L, Liu J, et al. LincRNA-p21: function and mechanism in cancer. Med Oncol 2017;34:98.

[64] Shi W, Wang Q, Bian Y, Fan Y, Zhou Y, Feng T, et al. Long noncoding RNA PANDA promotes esophageal squamous carcinoma cell progress by dissociating from NF-YA but interact with SAFA. Pathol Res Pract 2019;215:152604.

[65] Schultz LB, Chehab NH, Malikzay A, Halazonetis TD. p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. J Cell Biol 2000;151:1381–90.

[66] Craigie R, Bushman FD. HIV DNA integration. Cold Spring Harb Perspect Med 2012;2:a006890.

[67] Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, et al. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 2010;142:409–19.

[68] Brennan CM, Steitz JA. HuR and mRNA stability. Cell Mol Life Sci 2001;58:266–77.

[69] Abdelmohsen K, Pullmann R Jr, Lal A, Kim HH, Galban S, Yang X, et al. Phosphorylation of HuR by Chk2 regulates SIRT1 expression. Mol Cell 2007;25:543–57.

[70] Kawai T, Lal A, Yang X, Galban S, Mazan-Mamczarz K, Gorospe M. Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR. Mol Cell Biol 2006;26:3295–307.

[71] Wang W, Caldwell MC, Lin S, Furneaux H, Gorospe M. HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. EMBO J 2000;19:2340–50.

[72] Wang W, Furneaux H, Cheng H, Caldwell MC, Hutter D, Liu Y, et al. HuR regulates p21 mRNA stabilization by UV light. Mol Cell Biol 2000;20:760–9.

[73] Yoon JH, Abdelmohsen K, Srikantan S, Yang X, Martindale JL, De S, et al. LincRNA-p21 suppresses target mRNA translation. Mol Cell 2012;47:648–55.

[74] Barichievy S, Naidoo J, Mhlanga MM. Non-coding RNAs and HIV: viral manipulation of host dark matter to shape the cellular environment. Front Genet 2015;6:108.

[75] Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, et al. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. Nat Genet 2011;43:621–9.

[76] Wu H, Zheng J, Deng J, Zhang L, Li N, Li W, et al. LincRNA-uc002yug.2 involves in alternative splicing of RUNX1 and serves as a predictor for esophageal cancer and prognosis. Oncogene 2015;34:4723–34.

[77] Klase Z, Yedavalli VS, Houzet L, Perkins M, Maldarelli F, Brenchley J, et al. Activation of HIV-1 from latent infection via synergy of RUNX1 inhibitor Ro5-3335 and SAHA. PLoS Pathog 2014;10:e1003997.

[78] Huan C, Li Z, Ning S, Wang H, Yu XF, Zhang W. Long noncoding RNA uc002yug.2 activates HIV-1 latency through regulation of mRNA levels of various RUNX1 isoforms and increased tat expression. J Virol 2018;92. e01844-17.

[79] Li H, Chi X, Li R, Ouyang J, Chen Y. A novel lncRNA, AK130181, contributes to HIV-1 latency by regulating viral promoter-driven gene expression in primary CD4+ T cells. Mol Ther Nucleic Acids 2020;20:754–63.

[80] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, et al. MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003;22:8031–41.

[81] Friedman J, Cho WK, Chu CK, Keedy KS, Archin NM, Margolis DM, et al. Epigenetic silencing of HIV-1 by the histone H3 lysine 27 methyltransferase enhancer of Zeste 2. J Virol 2011;85:9078–89.

[82] Qu D, Sun WW, Li L, Ma L, Sun L, Jin X, et al. Long noncoding RNA MALAT1 releases epigenetic silencing of HIV-1 replication by displacing the polycomb repressive complex 2 from binding to the LTR promoter. Nucleic Acids Res 2019;47:3013–27.

[83] Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell 2009;33:717–26.

[84] Chao TC, Zhang Q, Li Z, Tiwari SK, Qin Y, Yau E, et al. The long noncoding RNA HEAL regulates HIV-1 replication through epigenetic Regulation of the HIV-1 promoter. mBio 2019;10:e02016–9.

[85] Yan MD, Hong CC, Lai GM, Cheng AL, Lin YW, Chuang SE. Identification and characterization of a novel gene Saf transcribed from the opposite strand of Fas. Hum Mol Genet 2005;14:1465–74.

[86] Boliar S, Gludish DW, Jambo KC, Kamng'ona R, Mvaya L, Mwandumba HC, et al. Inhibition of the lncRNA SAF drives activation of apoptotic effector caspases in HIV-1–infected human macrophages. Proc Natl Acad Sci U S A 2019;116:7431–8.

[87] Smith CM, Steitz JA. Classification of gas5 as a multi-small-nucleolar-RNA (snoRNA) host gene and a member of the 5′-terminal oligopyrimidine gene family reveals common features of snoRNA host genes. Mol Cell Biol 1998;18:6897–909.

[88] Chen L, Chen L, Zuo L, Gao Z, Shi Y, Yuan P, et al. Short communication: long noncoding RNA gas5 inhibits HIV-1 replication through interaction with miR-873. AIDS Res Hum Retroviruses 2018;34:544–9.

[89] Barclay RA, Schwab A, Demarino C, Akpamagbo Y, Lepene B, Kassaye S, et al. Exosomes from uninfected cells activate transcription of latent HIV-1. J Biol Chem 2017;292:11682–701.

[90] Pinto DO, Scott TA, Demarino C, Pleet ML, Vo TT, Saifuddin M, et al. Effect of transcription inhibition and generation of suppressive viral non-coding RNAs. Retrovirology 2019;16:13.

[91] Postler TS, Pantry SN, Desrosiers RC, Ghosh S. Identification and characterization of a long non-coding RNA up-regulated during HIV-1 infection. Virology 2017;511:30–9.

[92] Qi P, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. Mod Pathol 2013;26:155–65.

[93] Jin C, Peng X, Xie T, Lu X, Liu F, Wu H, et al. Detection of the long noncoding RNAs nuclear-enriched autosomal transcript 1 (NEAT1) and metastasis associated lung adenocarcinoma transcript 1 in the peripheral blood of HIV-1-infected patients. HIV Med 2016;17:68–72.

[94] Porro A, Feuerhahn S, Lingner J. TERRA-reinforced association of LSD1 with MRE11 promotes processing of uncapped telomeres. Cell Rep 2014;6:765–76.

[95] He B, Li W, Wu Y, Wei F, Gong Z, Bo H, et al. Epstein-Barr virus-encoded MIR-BART6-3p inhibits cancer cell metastasis and invasion by targeting long non-coding RNA LOC553103. Cell Death Dis 2016;7:e2353.

[96] Sethuraman S, Gay LA, Jain V, Haecker I, Renne R. microRNA dependent and independent deregulation of long non-coding RNAs by an oncogenic herpesvirus. PLoS Pathog 2017;13:e1006508.

[97] Zhang Z, Zhu Z, Watabe K, Zhang X, Bai C, Xu M, et al. Negative regulation of lncRNA GAS5 by miR-21. Cell Death Differ 2013;20:1558–68.

[98] Liang Y, Chen X, Wu Y, Li J, Zhang S, Wang K, et al. LncRNA CASC9 promotes esophageal squamous cell carcinoma metastasis through upregulating LAMC2 expression by interacting with the CREB-binding protein. Cell Death Differ 2018;25:1980–95.

[99] Meng L, Ward AJ, Chun S, Bennett CF, Beaudet AL, Rigo F. Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. Nature 2015;518:409–12.

[100] Smith C, Gore A, Yan W, Abalde-Atristain L, Li Z, He C, et al. Whole-genome sequencing analysis reveals high specificity of CRISPR/Cas9 and TALEN-based genome editing in human iPSCs. Cell Stem Cell 2014;15:12–3.

[101] Liu X, Homma A, Sayadi J, Yang S, Ohashi J, Takumi T. Sequence features associated with the cleavage efficiency of CRISPR/Cas9 system. Sci Rep 2016;6:19675.

[102] Kopp F, Elguindy MM, Yalvac ME, Zhang H, Chen B, Gillett FA, et al. PUMILIO hyperactivity drives premature aging of norad-deficient mice. Elife 2019;8:e42650.

[103] Pedram Fatemi R, Salah-Uddin S, Modarresi F, Khoury N, Wahlestedt C, Faghihi MA. Screening for small-molecule modulators of long noncoding RNA-protein interactions using alphascreen. J Biomol Screen 2015;20:1132–41.

[104] Mishra S, Verma SS, Rai V, Awasthee N, Chava S, Hui KM, et al. Long non-coding RNAs are emerging targets of phytochemicals for cancer and other chronic diseases. Cell Mol Life Sci 2019;76:1947–66.

[105] Xia D, Sui R, Zhang Z. Administration of resveratrol improved Parkinson's disease-like phenotype by suppressing apoptosis of neurons via modulating the MALAT1/miR-129/SNCA signaling pathway. J Cell Biochem 2019;120:4942–51.

[106] Saghafi T, Taheri RA, Parkkila S, Emameh RZ. Phytochemicals as modulators of long non-coding RNAs and inhibitors of cancer-related carbonic anhydrases. Int J Mol Sci 2019;20:2939.

[107] Liu G, Xiang T, Wu QF, Wang WX. Curcumin suppresses the proliferation of gastric cancer cells by downregulating H19. Oncol Lett 2016;12:5156–62.

[108] Ransohoff JD, Wei Y, Khavari PA. The functions and unique features of long intergenic non-coding RNA. Nat Rev Mol Cell Biol 2018;19:143–57.

[109] St Laurent G, Shtokalo D, Tackett MR, Yang Z, Eremina T, Wahlestedt C, et al. Intronic RNAs constitute the major fraction of the non-coding RNA in mammalian cells. BMC Genomics 2012;13:504.

[110] Nakaya HI, Amaral PP, Louro R, Lopes A, Fachel AA, Moreira YB, et al. Genome mapping and expression analyses of human intronic noncoding RNAs reveal tissue-specific patterns and enrichment in genes related to regulation of transcription. Genome Biol 2007;8:R43.

[111] St Laurent G, Wahlestedt C, Kapranov P. The Landscape of long noncoding RNA classification. Trends Genet 2015;31:239–51.

[112] Cary DC, Peterlin BM. Targeting the latent reservoir to achieve functional HIV cure. F1000Res 2016;5:F1000. Faculty Rev-1009.

[113] Elsheikh MM, Tang Y, Li D, Jiang G. Deep latency: a new insight into a functional HIV cure. EBioMedicine 2019;45:624–9.