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Discovery of long non-coding RNAs in naïve CD4⁺ T cells in response to initiating antiretroviral therapy at acute or chronic phase of HIV-1 infection

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Long non-coding RNAs (lncRNAs) have gained prominence due to their involvement in various cellular processes, but their specific roles remain elusive. Dysregulation of lncRNAs has been implicated in the pathogenesis of several diseases. In this study, we aimed to shed light on the role of lncRNAs in individuals infected with human immunodeficiency virus type 1 (HIV-1) by examining their changes in the expression patterns related to the initiation of antiretroviral therapy (ART) during acute or chronic phases of infection, compared to healthy controls. We found 316 differentially expressed (DE) lncRNAs in patients receiving long-term ART, shedding light on their potential roles. We also observed interactions between these DE lncRNAs and specific microRNAs (miRNAs). Some of these miRNAs, such as hsa-miR-574-5p, hsa-miR-765, hsa-miR-6165, hsa-miR-1207-5p, and hsa-miR-378i, are associated with cancer progression or suppression, while others, including hsa-miR-328-5p, hsa-miR-4753-3p, and MiR-664, play roles in immune system regulation. Furthermore, our study revealed substantial enrichment in distinct Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, such as axon guidance, PI3K-Akt signaling, and MAPK signaling pathways. Although our results indicate possible molecular processes impacted by the discovered lncRNAs, we cannot explicitly establish causality or specific connections between lncRNAs and genes in these pathways, fostering more specific studies. Furthermore, Gene Ontology (GO) analysis highlighted terms such as cytoskeletal protein binding, ion channel function, synaptic processes, neuron projection, and the somatodendritic compartment, underscoring the relevance of lncRNAs in these cellular components within the context of HIV-1 infection and ART treatment. In conclusion, our study emphasizes the need for further exploration of lncRNAs as potential biomarkers and therapeutic targets in HIV-1-infected patients, with a

particular focus on CD4⁺ T cells. Understanding the functions of lncRNAs in these contexts may pave the way for novel treatment strategies and improved patient outcomes, aligning with the broader goals of our research.

KEYWORDS

antiretroviral therapy, HIV-1, long non-coding RNA, RNA-seq, T-Lymphocytes

Introduction

Acquired immunodeficiency syndrome (AIDS), a disease caused by human immunodeficiency virus type 1 (HIV-1), is responsible for millions of deaths worldwide (De Boer et al., 2003; Margolis et al., 2020). The disease results in the susceptibility of the body to opportunistic infections. This occurs because of immunodeficiency caused by the death of CD4⁺ T cells infected with the HIV-1 virus (Campos Coelho et al., 2020). The current common treatment for HIV-1 consists of drugs that target viral enzymes, such as nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) and protease and integrase strand transfer inhibitors (PIs and INSTIs) (Bandera et al., 2019). Studies in infected humans have shown that antiretroviral therapy (ART) virtually reduces plasmatic viremia by stopping viral replication in the blood (Bale and Kearney, 2019; Bandera et al., 2019; Margolis et al., 2020). In the absence of ART, viral genome integration into host cell DNA occurs, leading to productive infection and the rapid death of CD4⁺ T cells (Leyre et al., 2020).

HIV-1 infection cannot be completely cured by ART, and discontinuing treatment at any time in a patient's life would lead to reactivation of the virus because HIV-1 mainly uses CD4⁺ memory T cells as a reservoir. The fact that these cells have a long half-life (nearly 500 days) helps the virus maintain latency in the host for life (De Boer et al., 2003; Chao et al., 2019; Campos Coelho et al., 2020). The timing of the onset of ART and the immunologic status of the individual are two of the most important factors in the risk of death in patients with HIV-1 infection (Chopra et al., 2019). In 2015, the World Health Organization (WHO) amended the criteria for introducing antiretroviral therapy (ART). These guidelines now propose that all people infected with HIV-1 should begin ART at the time of their HIV-1 diagnosis and potentially even during the acute infection phase. The primary objective of this recommendation is to prevent the deterioration of the immune system and the inflammation caused by the virus (Petkov and Chiodi, 2022). However, further studies are needed to determine how the mentioned factors affect the outcome of HIV-1 infection (Morcilla et al., 2021).

In recent years, scientists have been able to study eukaryotic genomes in greater detail. This has been made possible by the advantages of next-generation sequencing, clustered regularly interspaced short palindromic repeats gene editing, RNA deep sequencing (RNA-Seq), and other developments in high-throughput gene sequencing technologies (Ørom et al., 2010; Moran et al., 2012; Chen and Shan, 2020; Zou and Xu, 2020). The results of studies have highlighted that only a small fraction of the human genome (<2%) is responsible for protein coding, and the rest consists of non-coding genes that produce non-coding RNA transcripts (ncRNAs) (Ørom et al., 2010; Moran et al., 2012; Zou and Xu, 2020). LncRNAs are transcripts (>200 nts) that lack protein-coding RNA. It has been demonstrated that lncRNAs play a critical role in the regulation of various cellular pathways, including viral diseases such as HIV-1 (Boliar et al., 2019).

An important functional area in which lncRNAs may have an impact is the host response to infectious particles such as viruses by regulating host responses and participating in the primary immune response (Peng et al., 2010; Wang et al., 2019). Research on lncRNAs could be of great benefit as they can alter disease progression, infection, and host response. Studying the function of lncRNAs may provide us with new biological indicators to track the progression of various diseases. Identifying new lncRNAs that are differentially expressed as a cause of viral infection may bring us one step closer to understanding their functions. In this study, we investigated new lncRNAs in HIV-1-infected patients and studied the difference between the expression of lncRNAs associated with the time when ART treatment was started and controls.

Materials and methods

Data selection

We investigated whole transcriptome data or total RNA-Seq studies. Sequence datasets were obtained from the GenBank data libraries[1] under accession number PRJNA724934 (Petkov and Chiodi, 2021). Thirty-nine RNA-seq datasets in FASTQ format were downloaded using the dataset sample experiment.

lncRNA discovery

De novo assembly of datasets

Datasets were analyzed using CLC Genomics Workbench (v20) software. All 39 datasets underwent quality control and

1 https://www.ncbi.nlm.nih.gov/genbank/

trimming. Additionally, reads less than 15 bp were automatically discarded through adapter trimming. The trimmed datasets were then *de novo* assembled, with a contig length threshold of 250 bp and a word size of 20.

Reference mapping

The human reference genome (GCF_000001405.40_GRCh38.p14_genomic) was obtained from NCBI[2]. The *de novo* assembly results were mapped against the annotated mRNA track of the reference genome using a length fraction of 0.8% and 95% identity. The unmapped reads were utilized for further analyses.

PFAM domain search

The unmapped contigs were translated into proteins using all six frames. Subsequently, the amino acid sequences underwent screening for PFAM domains, and any identified protein domain was subsequently excluded from the obtained data. The conserved protein domains were identified using the Pfam domain search algorithm used in the CLC Genomic Workbench.

ORF finding

The PFAM domain search results were uploaded to CPC[3], and the encoding potential was determined. All contigs with coding potential were discarded, and the remaining contigs were used for further analyses.

BLASTx against protein databases

A complete protein database was created in the CLC Genomics Workbench (v20) using all the existing protein databases for humans and animals in NCBI and UniProt[4]. The BLASTx algorithm was used to check the similarity of the reads with known proteins (query genetic code: 1 standard, E-value <0.0001 and a maximum number of hit sequences = 20, word size: 3, matrix: BLOSUM62, gap cost: existence 11, extension 1, and a maximum number of hit sequences: 2). The complete animal protein RefSeq and PFAM databases were also used to find protein-coding reads. All the remaining contigs that were identified as proteins were sorted out. The remaining contigs were considered putative lncRNAs.

Annotation of putative lncRNAs

In order to distinguish novel lncRNAs from conserved lncRNAs, the FASTA format of the human lncRNA reference (Homo_sapiens.GRCh38) was downloaded from Ensembl[5]. Subsequently, the putative lncRNAs were annotated with the lncRNA reference sequence using the BLASTn program (*p-value* < 0.0001). Finally, a new lncRNA reference was created, consisting of both the conserved and novel lncRNAs. The lncRNA discovery method is summarized in Figure 1.

Expression and statistical analysis

In order to determine which lncRNAs were up- and downregulated in each sample, the previously generated trimmed datasets were mapped against the newly generated lncRNA reference (length fraction = 0.8, similarity fraction = 0.8, mismatch cost = 2, insertion cost = 3, deletion cost = 3, and the maximum number of hits for a read = 10. Expression values were determined as reads per kilobase of transcript per million reads mapped, RPKM) (Davati and Ghorbani, 2023). The mapped results for each sample were used for expression analysis. The expression analysis results were statistically analyzed using Empirical Analysis DGE. The analysis included the exact test comparison, and samples were compared to controls using FDR-corrected *p*-values. The data were then filtered to identify differentially expressed (DE) lncRNAs. LncRNAs with an EDGE test fold change (FC) of < -2 or >2 and an EDGE test FDR *p*-value correction <0.05 were maintained as DE lncRNAs. A heatmap was generated using heatmapper tools[6] with default parameters (average linkage clustering methods and Euclidean distance measurement methods) to calculate Z-Score from the RPKMs of lncRNAs in samples.

LncRNA-miRNA interaction analysis

The mature miRNA sequences of *Homo sapiens* were extracted from the miRBase database[7]. Lnc-miRNA interactions were determined using psRNATarget[8]. Interaction

2 http://www.ncbi.nlm.nih.gov/
3 http://cpc2.gao-lab.org/
4 https://www.uniprot.org/
5 https://useast.ensembl.org/index.html
6 http://www.heatmapper.ca/expression/
7 https://www.mirbase.org/
8 www.zhaolab.org

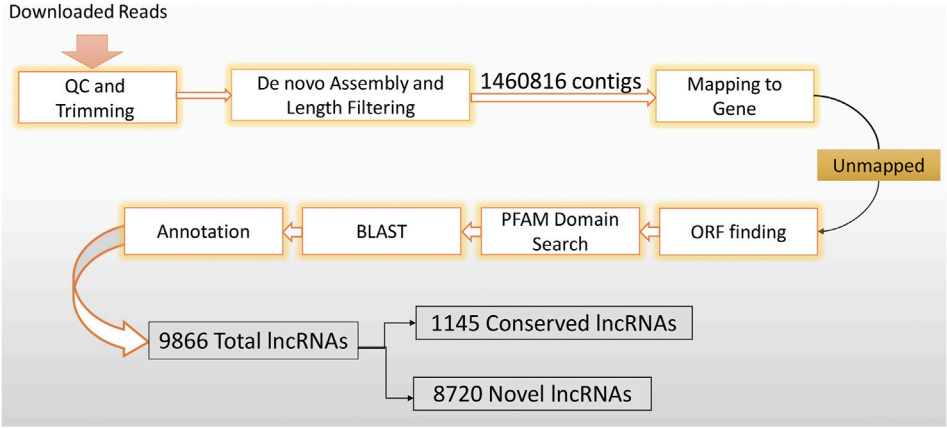

FIGURE 1
LncRNA discovery method flowchart. In order to get highly confident putative lncRNAs, downloaded datasets were imported into the CLC genomic workbench and subjected to rigorous filtering.

networks for commonly up- and downregulated lncRNAs with mature miRNAs were then generated using Cytoscape (v3.9.1) software[9].

Identification of the subcellular localization of lncRNAs

LncRNA sequences for the two common groups (upregulated and downregulated) were uploaded in FASTA format into LightGBM-LncLoc (LncRNA subcellular localization predictor based on LightGBM) (Lyu et al., 2023).

mRNA-lncRNA interaction and gene ontology analysis

The chromosome localization of upregulated and downregulated lncRNAs was determined using Geneious Prime (v.2019.1)[10] for transcript annotation of ncRNA. The annotated sequences were further aligned with the human chromosome reference sequence utilizing the Blast annotation tools (with a maximum E-value of ≤0.0001). Subsequently, the results were converted to tracks using the track conversion tool. These track data were manually analyzed to identify genomic regions wherein genes were located adjacent to the lncRNAs, considering a distance of 10 kb. This manual analysis permitted a more detailed assessment of the relationships between lncRNAs and neighboring genes, leading to the gaining of multiple chromosomal regions.

Ensembl BioMart[11] was important in transforming the numerous chromosomal regions acquired into matching gene IDs. This phase improved the accuracy and organized classification of the genes linked to the investigated lncRNAs.

The discovered genes were analyzed for enrichment in gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The STRING v.11.5[12] was used to do multiple protein searches in the human database, using the default parameters.

Results and discussion

Identification of putative lncRNAs in human CD4$^+$ naïve T cells

A strong reference of human CD4$^+$ naïve T-cell lncRNAs was generated using 39 datasets (Table 1) extracted from SRA. These datasets were previously collected for another study examining the effects of using ART in the early stages of infection on CD4$^+$ naïve T cells. Overall, the RNA-seqs included 13 datasets from patients who received early ART (EA), 11 datasets from patients who received late ART (LA), and 15 datasets from healthy controls. To identify lncRNAs, we developed a highly restricted methodology. Briefly, 1,460,816 contigs were detected, consisting of coding and non-coding transcripts.

9 https://cytoscape.org/
10 https://www.geneious.com/
11 http://asia.ensembl.org/biomart/martview
12 https://string-db.org/

TABLE 1 List of datasets.

Sample number	The accession number of data	Group	Total read count
1	SRX10675595	control	34,169,654
2	SRX10675594	LA	55,268,248
3	SRX10675593	LA	86,378,288
4	SRX10675592	LA	44,439,994
5	SRX10675591	LA	35,010,748
6	SRX10675590	EA	78,017,330
7	SRX10675589	LA	72,410,186
8	SRX10675588	LA	99,308,734
9	SRX10675587	LA	51,682,766
10	SRX10675586	LA	79,244,324
11	SRX10675585	LA	69,689,758
12	SRX10675584	LA	71,317,638
13	SRX10675583	LA	150,920,454
14	SRX10675582	EA	114,581,860
15	SRX10675581	EA	44,737,142
16	SRX10675580	EA	81,083,364
17	SRX10675579	EA	81,657,722
18	SRX10675578	EA	76,240,072
19	SRX10675577	EA	67,252,994
20	SRX10675576	EA	37,951,460
21	SRX10675575	EA	62,924,538
22	SRX10675574	EA	65,524,846
23	SRX10675573	EA	114,245,216
24	SRX10675572	EA	127,145,206
25	SRX10675571	control	69,842,752
26	SRX10675570	control	61,941,916
27	SRX10675569	control	92,125,548
28	SRX10675568	control	117,153,850
29	SRX10675567	control	112,315,928
30	SRX10675566	control	61,425,042
31	SRX10675565	control	83,418,752
32	SRX10675564	control	57,088,636
33	SRX10675563	control	101,202,264
34	SRX10675562	EA	64,192,032
35	SRX10675561	control	51,698,702
36	SRX10675560	control	59,792,818

(Continued on following page)

TABLE 1 (*Continued*) List of datasets.

Sample number	The accession number of data	Group	Total read count
37	SRX10675559	control	59,959,538
38	SRX10675558	control	114,663,024
39	SRX10675557	control	51,763,856

Thirty-nine datasets were used for this study. Datasets are separated into three groups. Early ART (EA), Late ART (LA), and control group.

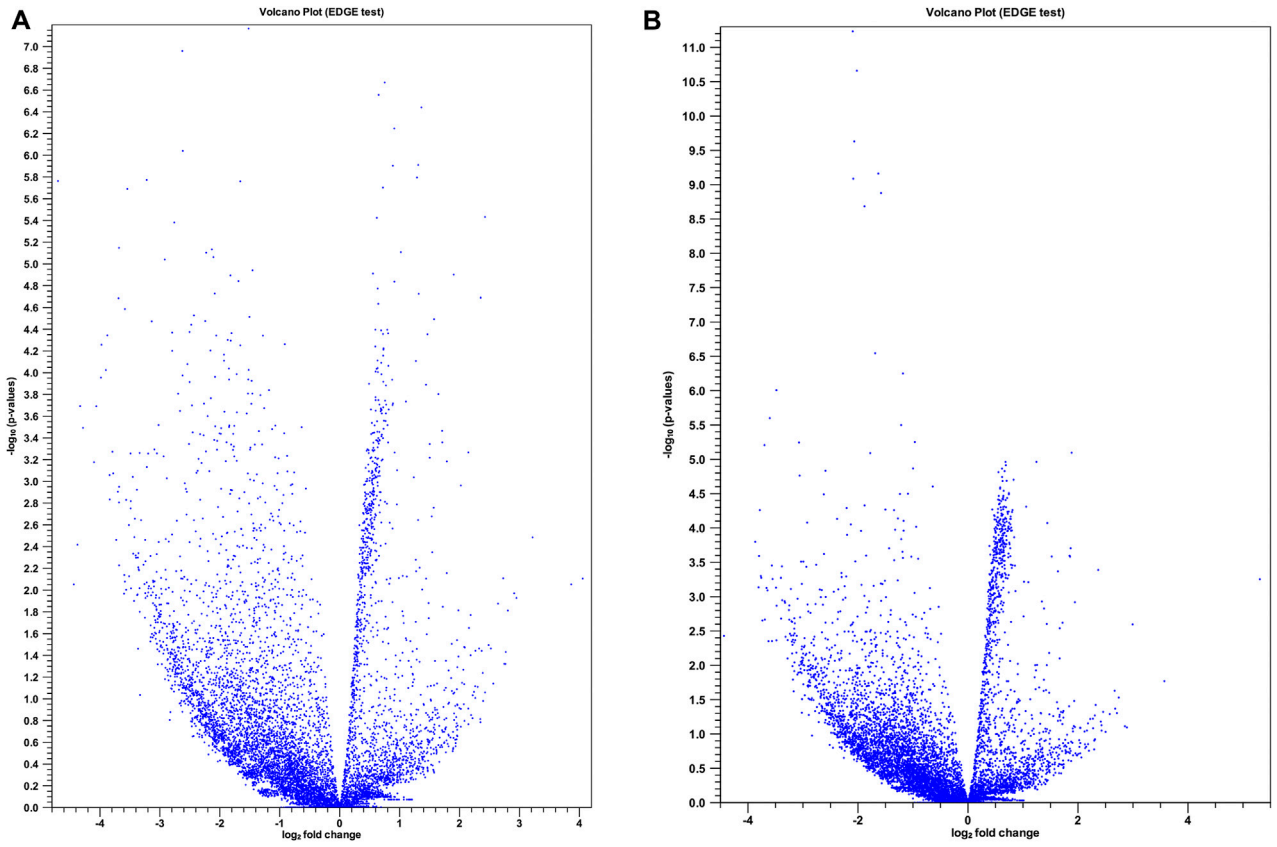

FIGURE 2
Volcano plots for differentially expressed (DE) lncRNAs in two treatment groups compared with control. **(A)** Control vs. late ART (LA) and **(B)** control vs. early ART (EA) with extraction and analysis of differential gene expression (EDGE) test. The *x*-axis shows the magnitude (log_2 fold change) of DE lncRNAs and the *y*-axis shows significance (– log 10 adjusted *p*-value). Each blue spot represents a specific DE lncRNA.

After removing the reads related to the genes, coding potential, or protein domains, the remaining reads with protein-coding ability were also discarded. Finally, a total of 9,866 lncRNAs were discovered. Annotation distinguished 8,720 novel lncRNAs from 1,145 conserved lncRNAs. In a previous study, 17,235 lncRNAs were identified from peripheral blood mononuclear cells (PBMCs) from three EA and three healthy controls (Ma et al., 2021). The difference between the identified lncRNAs is likely due to the rigorous lncRNA discovery method we used and, more importantly, the fact that in our study, lncRNAs were identified only for naïve $CD4^+$ T cells purified from PBMCs.

Differential expression of lncRNAs in EA and LA groups compared to control

To gain a comprehensive understanding of differentially expressed (DE) lncRNAs in $CD4^+$ T cells during treated HIV-1 infection, we compared 13 EA and 11 LA RefSeq

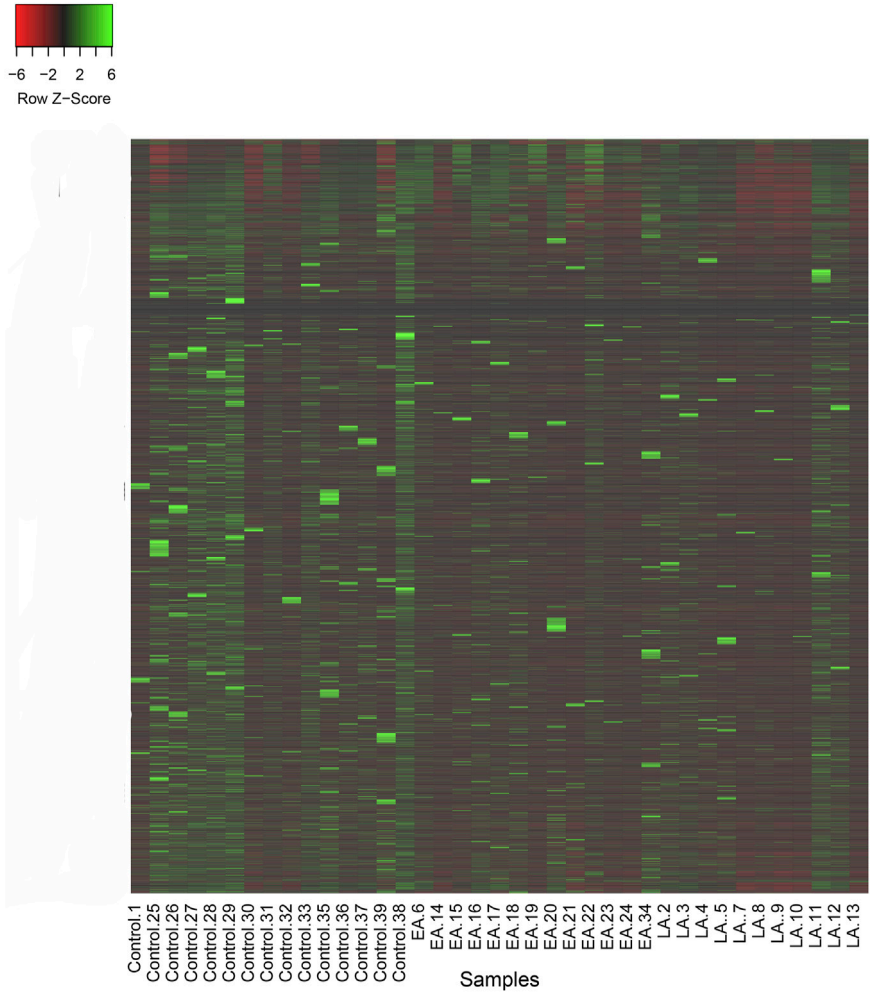

FIGURE 3
Heatmap of reads per kilobase of transcript per million reads mapped (RPKM) for lncRNAs in two treatment groups, late ART (LA) and early ART (EA), and control. Higher values of Z-Score for lncRNAs in each sample are shown in green and lower values are shown in red. Z-Score is calculated by: (RPKM in a sample of interest) - (Mean RPKM across all samples)/Standard Deviation.

transcriptomic datasets with 15 controls. Supplementary Data S1 includes an analysis of DE lncRNAs in both the EA and LA groups compared to the controls. After filtering, we confirmed that 20 lncRNAs were upregulated in the EA group, while 122 were downregulated compared to the control group. In the LA group, 23 lncRNAs were upregulated, and 151 were downregulated compared to the control group. We identified 20 downregulated and 9 upregulated lncRNAs that were common to both groups (EA and LA). Volcano plots for each group are shown in Figure 2 and a heat map for lncRNAs is provided in Figure 3. Ma et al. identified 36 upregulated and 206 downregulated lncRNAs in PBMCs from three ART-naive patients with early HIV-1 infection (within 180 days of HIV-1 infection) and three healthy controls (Ma et al., 2021). Trypsteen et al. detected 262 differentially expressed lncRNAs in cultured central memory T cells and bystander models compared to mock-infected cells. The majority of dysregulated lncRNAs were upregulated in both models (Trypsteen et al., 2019). Based on microarray analysis, Nair et al. identified 795 lncRNAs that were downregulated during active infection in U-937 monocytes infected with HIV-1 (*in vitro*) and 389 lncRNAs that were upregulated compared to uninfected controls (Nair et al., 2016). Our results are consistent with two of the mentioned studies regarding the expression pattern of DE lncRNAs. We also observed a higher number of downregulated lncRNAs compared to upregulated ones, which aligns with their findings. The difference in the number of DE lncRNAs observed in our study may be attributed to the influence of antiretroviral therapy (ART) on lncRNA expression, particularly in the LA group, where patients had undergone ART for an extended period. Furthermore, recent evidence suggests that the expression of lncRNAs is cell-type specific (Bjørklund et al., 2022).

TABLE 2 Top upregulated lncRNAs in three groups compared with the control group.

Transcript no.	Name	Fold change	FDR *p-value*
EA 1	hu-lnc780	39.57168484	0.017237619
EA 2	hu-lnc187	7.961527584	0.049157182
EA 3	hu-lnc2913	5.160555332	0.014200997
EA 4	hu-lnc8565	3.846691366	0.02882841
EA 5	hu-lnc5646	3.692370046	0.004703611
LA 1	ENST00000543611.1	5.36994557	0.002181244
LA 2	hu-lnc2488	5.105537004	0.005826146
LA 3	hu-lnc8565	4.427740065	0.026279363
LA 4	hu-lnc3713	4.059190683	0.037350128
LA 5	hu-lnc5646	3.737612717	0.004487648
common 1	hu-lnc8565		
common 2	hu-lnc5646		
common 3	hu-lnc3019		
common 4	ENST00000543611.1		
common 5	hu-lnc3816		

Early ART (EA), Late ART (LA), and commonly upregulated lncRNAs to both groups.

TABLE 3 Top downregulated lncRNAs in three groups compared with the control group.

Transcript number	Name	Fold change	FDR *p-value*
EA 1	hu-lnc4290	−14.60018718	0.008895827
EA 2	hu-lnc3327	−13.99857973	0.020534228
EA 3	hu-lnc2242	−13.91309927	0.010734037
EA 4	hu-lnc8696	−13.77565907	0.00778841
EA 5	hu-lnc770	−13.60539735	0.016151879
LA 1	hu-lnc5224	−25.99547062	0.001317313
LA 2	hu-lnc501	−20.11873906	0.017493752
LA 3	hu-lnc4993	−19.46634416	0.02170685
LA 4	hu-lnc1766	−17.13418977	0.028832012
LA 5	hu-lnc1632	−16.68919228	0.017493752
common 1	hu-lnc1632		
common 2	hu-lnc3267		
common 3	hu-lnc1718		
common 4	hu-lnc3103		
common 5	hu-lnc85		

Early ART (EA), Late ART (LA), and commonly upregulated lncRNAs to both groups (EA and LA).

We restricted our study to naïve CD4$^+$ T cells, which could be another reason for the differences in the number of differentially expressed (DE) lncRNAs. Additionally, some of the studies were performed *in vitro*, which could also influence the outcome. Two top-upregulated lncRNAs (hu-lnc5646 and hu-lnc8565) (Tables 2, 3) were common in both the EA and LA groups. Further investigation of these lncRNAs may provide us with biomarkers for HIV-1 infection. ENST00000543611.1 was the only conserved lncRNA among the top upregulated lncRNAs (Table 2). Among the identified lncRNAs, some belonged to already known lncRNAs such as ENTPD1 antisense RNA 1 (ENST00000665049.1), TRIM8 divergent transcript (ENST00000607967.1), and PRR34 antisense RNA 1 (ENST00000451166.5). More information on the DE lncRNAs is provided in Supplementary Data S1. One of the commonly upregulated lncRNAs is ENST00000661141.1, also known as long intergenic non-protein coding RNA 1278 (LINC01278). LINC01278 may play different roles in different tumors. One study indicated that LINC01278 contributes to the development of hepatocellular carcinoma (HCC) (Huang et al., 2020). Another study found that LINC01278, as a positive regulator of DNM3 expression, suppresses thyroid carcinoma progression (Lin et al., 2019). Although it is not clear how LINC01278 functions in HIV-1 positive patients, the upregulation of this lncRNA in both treatments seems to play a role in human response to ART. Hu-lnc780 in the EA group (Table 2) showed a significantly greater extent of upregulation, whereas hu-lnc5224 in the LA group (Table 3) exhibited a much greater extent of downregulation, making them attractive candidates for further investigation.

LncRNAs affect miRNAs involved in tumor regulation

The extensive repertoire of miRNAs and their wide-ranging capacity to target various genes indicate the presence of an intricate regulatory network that modulates gene expression (Disner et al., 2021). Therefore, the mature miRNA sequences of *H. sapiens* were acquired from the miRBase database to anticipate these interactions. Then, the up-and downregulated lncRNAs for each treatment and the common up- and downregulated lncRNAs in both treatments were identified. Each group was submitted to psRNATarget[8] for comparison with miRNA sequences from miRBase. Interaction networks of common up- and downregulated lncRNAs and mature miRNAs were then generated using Cytoscape software (v3.9.1). The top 50 interactions were selected to allow for more detailed visualization. Figure 4 shows the top 50 common lncRNA-miRNA interaction networks. A highly upregulated lncRNA (hu-lnc8565) was found to interact with at least nine different miRNAs (Figure 4A), including hsa-miR-4739, hsa-miR-6873-3p, hsa-miR-6838-3p, hsa-miR-4753-3p, hsa-miR-6501-5p, hsa-miR-6090, hsa-miR-6796-5p, hsa-miR-6085, and hsa-miR-6891-5p. ENST00000543611.1 was another highly upregulated lncRNA that showed interactions with several miRNAs (Figure 4A), such as hsa-miR-3911, hsa-miR-3147, hsa-miR-6640-5p, hsa-miR-4539, hsa-miR-6800-5p, and hsa-miR-6779-5p. A highly downregulated lncRNA (hu-lnc1632) also showed interactions with at least two miRNAs, including hsa-miR-6875-3p and hsa-miR-574-5p (Figure 4B). The results also showed that key miRNAs, including hsa-miR-574-5p, hsa-miR-765, hsa-miR-6165, and hsa-miR-1207-5p, can be controlled by commonly downregulated lncRNAs. Most of these miRNAs are thought to have targets involved in tumor prevention or development. Hsa-miR-574-5p has been shown to reduce liver metastasis in colon cancer by negatively regulating the expression of Metastasis-Associated in Colon Cancer 1, a biomarker of carcinogenesis and metastasis in more than 20 cancers (Cui et al., 2014; Wen et al., 2020). However, hsa-miR-574-5p was found to be a major factor associated with accelerated tumor growth in human lung cancer (Li et al., 2012). Hsa-miR-765, which is overexpressed in many human cancers, may act as a tumor suppressor and control arterial stiffness (Xie et al., 2016; Pallez et al., 2017; Zammit et al., 2018). It has been shown that hsa-miR-765 can increase cell growth by suppressing the expression of INPP4B in HCC (Liu et al., 2017). Hsa-miR-6165 is expressed in brain tumor-derived cell lines and primary brain tumor tissues. Its overexpression has been shown to downregulate the apoptosis-related genes PKD1 and DAGLA and induce NGFR, which functions as a tumor suppressor in most cancers (Parsi et al., 2012). Hsa-miR-1207-5p prevents gastric cancer development and invasion by targeting telomerase reverse transcriptase, ultimately leading to tumor senescence and death (Chen et al., 2014; Xu et al., 2015). It interacts with the 3′UTR of hTERT in gastric malignancies and is significantly downregulated in tissue samples from these tumors (Qin et al., 2015; Dinami et al., 2017). Moreover, hsa-miR-1207-5p was found to be strongly downregulated in breast cancer compared with healthy tissues (Dinami et al., 2017). Frequently upregulated lncRNAs also show interactions with important miRNAs such as hsa-miR-378i, hsa-miR-328-5p, hsa-miR-4753-3p, and hsa-miR-664b-5p. Hsa-miR-378i may act as a negative regulator in the progression of colon cancer by inducing apoptosis and preventing colon cancer cells from migrating and invading other tissues (Zeng et al., 2017).

However, hsa-miR-378i has also been shown to increase cell survival, decrease caspase-3 activity, and promote tumorigenesis, angiogenesis, and neurogenesis. It can also induce neuroprotection by negatively regulating caspase-3-linked apoptosis (Meganathan et al., 2015; Zhang et al., 2016). The expression of hsa-miR-328-5p is closely associated with cell cycle progression, and numerous malignancies exhibit low expression of hsa-miR-328-5p (Wu et al., 2012). It has been established that hsa-miR-328-5p plays a role in the control of phagocytosis and bacterial survival, and its downregulation increases the clearance of pathogens in the lung. Downregulation of hsa-miR-4753-3p has been shown to target genes involved in immunologically significant signaling pathways, such as MAPK signaling, cytokine-cytokine receptor interactions, endocytosis, actin

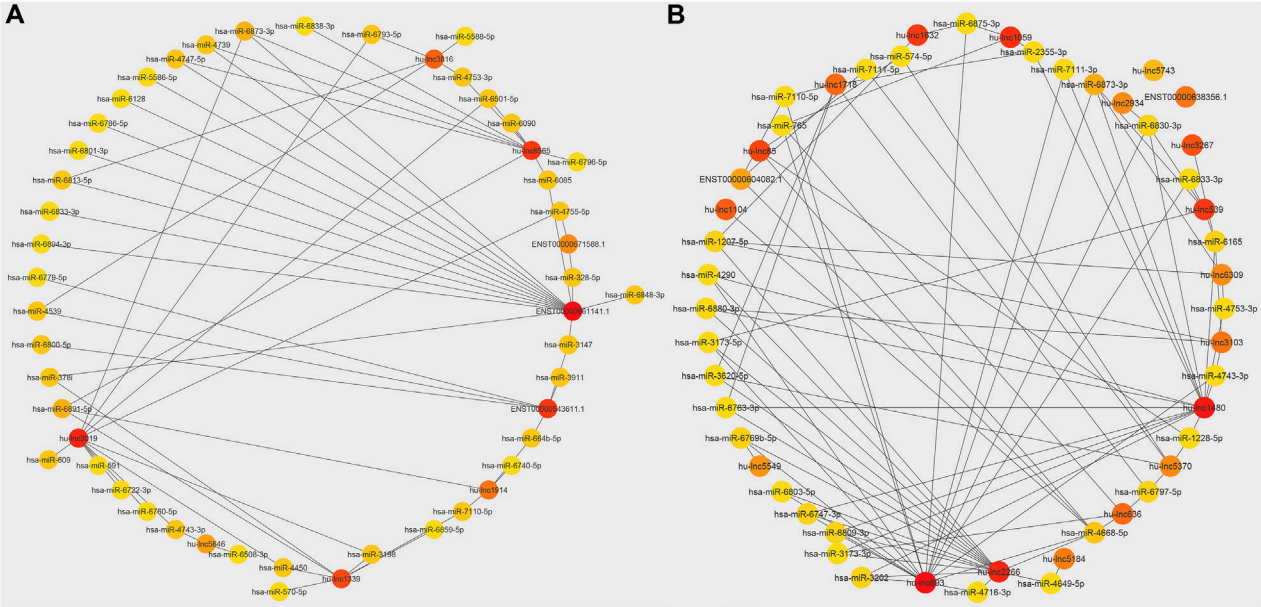

FIGURE 4
Network of interaction between top common lncRNAs and miRNAs. **(A)** Commonly upregulated lncRNAs and miRNAs interaction. **(B)** Commonly downregulated lncRNAs and miRNAs interaction. The colors of the circles, red, orange, and yellow, indicate the importance of interaction nodes ranging from high to low.

cytoskeleton regulation, focal adhesion, calcium signaling, chemokine signaling, and Wnt signaling (Budak et al., 2018). MiR-664 may function as a pro-viral miRNA that is essential for the virus to downregulate downstream target genes involved in viral defense systems in the host cell (Wolf et al., 2016). Given this information, it is clear that these miRNAs can alter the course of the disease, play an important role in the immune response, and affect cancer cells. The interaction between lncRNAs expressed in patients who have received long-term ART with these miRNAs suggests that ART can affect controlling cancer and regulating the immune system. The findings are important because ART therapeutics, such as protease inhibitors (PIs), have been proven to have potent antiangiogenic effects and cause regression of Kaposi sarcoma (Sgadari et al., 2002; Toschi et al., 2011). However, the exact role of lncRNAs in regulating miRNAs and how these interactions may affect cancer or the immune system remains unclear and requires further research.

Identification of the subcellular localization of lncRNAs

As with proteins, the function of lncRNAs depends on their subcellular localization (Chen, 2016). The subcellular microenvironment enables different functions of the same lncRNA by allowing interactions with different functional protein partners and action targets (Bridges et al., 2021). The results of our study showed that the most common DE lncRNAs were localized in the nucleus. Eight out of twenty common downregulated lncRNAs were localized in the nucleus, and twelve lncRNAs were located in the cytoplasm (Table 4). Furthermore, eight out of nine common upregulated lncRNAs were localized in the nucleus, and one lncRNA was localized in the ribosome (Table 5). In total, 16 lncRNAs were localized in the nucleus, 12 in the cytoplasm, and one lncRNA in the ribosome. Structurally, lncRNAs are preferentially localized in the nucleus, more tissue-specific, less highly expressed, and evolutionarily less conserved than mRNAs (Liu et al., 2022).

When localized in the nucleus, lncRNAs are involved in gene regulation at the epigenetic and transcriptional levels, including histone modifications, regulation of DNA methylation and chromatin remodeling, interaction with chromatin modification complexes, transcription factors, and proteins in the nucleus (Xing et al., 2021). Previous studies have shown that the substructural integrity of the nuclear paraspeckle, an important subcellular organelle for HIV-1 replication, is highly dependent on a lncRNA called NEAT1 (Zhang et al., 2013). The second group of lncRNAs must be exported to the cytoplasm to fulfill their regulatory roles (Chen, 2016). LncRNAs localized in the cytoplasm often function as scaffolds for protein complexes or as sponges for miRNAs acting at

TABLE 4 Distribution of subcellular localization of commonly downregulated lncRNAs to both groups (EA and LA).

ID	Result	Cytoplasm	Nucleus	Ribosome	Cytosol	Exosome
ENST00000604082.1	Cytoplasm	0.762	0.235	0.003	0	0
ENST00000638356.1	Cytoplasm	0.818	0.182	0	0	0
hu-lnc85	Nucleus	0.427	0.573	0	0	0
hu-lnc539	Nucleus	0.134	0.865	0	0.001	0
hu-lnc636	Nucleus	0.437	0.563	0	0	0
hu-lnc693	Nucleus	0.37	0.623	0.003	0.004	0
hu-lnc1059	Cytoplasm	0.693	0.301	0.005	0.001	0
hu-lnc1104	Cytoplasm	0.508	0.491	0	0	0
hu-lnc1480	Cytoplasm	0.638	0.361	0	0	0
hu-lnc1632	Cytoplasm	0.842	0.158	0	0	0
hu-lnc1718	Cytoplasm	0.512	0.266	0.213	0.009	0
hu-lnc2266	Nucleus	0.106	0.894	0	0	0
hu-lnc2934	Nucleus	0.32	0.678	0.001	0.001	0
hu-lnc3103	Cytoplasm	0.604	0.396	0	0	0
hu-lnc3267	Cytoplasm	0.606	0.392	0	0.002	0
hu-lnc5184	Nucleus	0.051	0.808	0.139	0.001	0
hu-lnc5370	Nucleus	0.303	0.696	0.001	0.001	0
hu-lnc5549	Cytoplasm	0.54	0.458	0.002	0	0
hu-lnc5743	Cytoplasm	0.585	0.414	0	0	0
hu-lnc6309	Cytoplasm	0.598	0.401	0.001	0	0

The numbers show the abundance of lncRNAs in subcellular organelles out of 1, concluding the potential localization in the result column.

TABLE 5 Distribution of subcellular localization in commonly upregulated lncRNAs to both groups (EA and LA).

ID	Result	Cytoplasm	Nucleus	Ribosome	Cytosol	Exosome
ENST00000671588.1	Nucleus	0.293	0.706	0	0	0.001
ENST00000661141.1	Nucleus	0.159	0.837	0	0.003	0
ENST00000543611.1	Nucleus	0.222	0.695	0	0	0.083
hu-lnc1339	Nucleus	0.348	0.652	0	0	0
hu-lnc1914	Nucleus	0.094	0.887	0.019	0.001	0
hu-lnc3019	Nucleus	0.11	0.889	0	0.001	0
hu-lnc3816	Nucleus	0.291	0.707	0	0.001	0
hu-lnc5646	Nucleus	0.407	0.591	0.001	0.001	0
hu-lnc8565	Ribosome	0.418	0.129	0.45	0.003	0

The numbers show the abundance of lncRNAs in subcellular organelles out of 1, concluding the potential localization in the result column.

posttranscriptional and translational levels (Chen, 2016; Shen et al., 2020). The results may suggest that in HIV-1-positive patients receiving ART, both types of subcellular localization are expressed, affecting both nucleus mechanisms and cytoplasm mechanisms. The pathways by which lncRNAs function in the different subcellular localizations and whether these actions are

TABLE 6 KEGG pathways analysis (*p-value* < 0.05) of the determined DE lncRNA neighboring genes using STRING ver. 11.5[12].

Category	Term ID	Term description	Observed gene count	Expected	*p-value*
KEGG	hsa04151	PI3K-Akt signaling pathway	35	350	0.0398
KEGG	hsa04010	MAPK signaling pathway	32	288	0.0066
KEGG	hsa04360	Axon guidance	22	177	0.0139
KEGG	hsa04020	Calcium signaling pathway	21	193	0.0427
KEGG	hsa04724	Glutamatergic synapse	17	111	0.0319
KEGG	hsa05032	Morphine addiction	16	89	0.0188
KEGG	hsa05412	Arrhythmogenic right ventricular cardiomyopathy	15	76	0.006
KEGG	hsa04713	Circadian entrainment	15	92	0.0139
KEGG	hsa04925	Aldosterone synthesis and secretion	15	95	0.0139
KEGG	hsa05414	Dilated cardiomyopathy	14	95	0.0289
KEGG	hsa04911	Insulin secretion	12	82	0.0498
KEGG	hsa04929	GnRH secretion	11	63	0.0289

The data is sorted based on the highest observed gene count.

beneficial for patient treatment are questions that remain unanswered.

GO enrichment analysis of the DE lncRNA-neighboring coding genes

We sought to investigate the potential targets of DE lncRNAs in cis-regulatory elements to shed light on their putative roles. Our analysis focused on identifying target genes of DE lncRNAs in $CD4^+$ T cells from HIV-1 infected + ART-treated patients compared to controls. We acknowledge that our research included a total of 20 downregulated lncRNAs and 9 upregulated lncRNAs out of 9,866 lncRNAs which were down and upregulated as common in both treatments (Supplementary Data S1). We identified 4,209 transcripts in DE lncRNA-neighboring.

Subsequently, Gene Ontology (GO) analysis was performed to explore the functional implications of transcripts in DE lncRNA-neighboring. Comprehensive details on the terms enriched in the GO are available in Supplementary Data S2.

In our analysis, we identified enrichment (*p-value* < 0.05), in twelve Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Table 6). Notably, three major KEGG pathways were significantly enriched, namely, axon guidance (*p-value* = 0.0139), PI3K-Akt signaling pathway (*p-value* = 0.0398), and MAPK signaling pathway (*p-value* = 0.0066). Previous studies have linked impairment of host axon guidance and these signaling pathways to neurological manifestations in HIV-1 patients (Zhou et al., 2012; Scutari et al., 2017; Ojeda-Juárez and Kaul, 2021). In addition, our analysis identified terms linked to signaling pathways related to neurodegenerative diseases, including the MAPK signaling pathway, axon guidance pathway, and phosphorylative mechanism/signaling pathway. This supports the concept that these pathways play a noteworthy role in the development of HIV-1-associated dementia (HAD) (Zhou et al., 2012). Furthermore, it is important to note that earlier research has shown that some initial HIV-1 protease inhibitors might hinder the activation of Akt, indicating a possible connection to the pathways we discovered in our investigation (Plastaras et al., 2008). However, it is essential to use caution when interpreting this outcome, and more research is necessary to establish a definitive association and understand the broader implications of these findings.

Notably, our study revealed a significant enrichment of the glutamatergic synapse pathway (*p-value* = 0.0319). This observation suggests a potential association between $CD4^+$ T cells and brain synaptic plasticity, which may have broader implications for cognitive functions (Zarif et al., 2018). It is crucial to accept that while our findings indicate a potential connection, more investigation is necessary to prove a cause-and-effect relationship. Prior research has shown that HIV-1 and viral proteins can interfere with the transmission of glutamate at synapses, which might lead to cognitive impairment (Ru and Tang, 2017; Gorska and Eugenin, 2020). Additionally, recent research has hinted at potential additive negative effects on the glutamatergic system when ART is combined with HIV-1 infection, further suggesting a connection to neuronal signaling (Gorska and Eugenin, 2020). These connections need thoughtful examination and deserve more experimental verification to completely understand their importance. While

TABLE 7 GO analysis (Biological Function) (*p-value* < 0.05) of the determined DE lncRNA neighboring genes using STRING ver. 11.5[12].

Category	Term ID	Term description	Observed gene count	Expected	*p-value*
Biological Function	GO:0005515	Protein binding	405	7,026	0.009
Biological Function	GO:0019899	Enzyme binding	146	2,239	0.038
Biological Function	GO:0030234	Enzyme regulator activity	80	1,044	0.0191
Biological Function	GO:0008092	Cytoskeletal protein binding	77	973	0.0133
Biological Function	GO:0015318	Inorganic molecular entity transmembrane transporter activity	63	813	0.0453
Biological Function	GO:0008324	Cation transmembrane transporter activity	55	629	0.0134
Biological Function	GO:0022890	Inorganic cation transmembrane transporter activity	54	584	0.0074
Biological Function	GO:0005216	Ion channel activity	45	425	0.0046
Biological Function	GO:0046873	Metal ion transmembrane transporter activity	44	427	0.0071
Biological Function	GO:0022836	Gated channel activity	40	332	0.0024
Biological Function	GO:0005261	Cation channel activity	38	326	0.0046
Biological Function	GO:0060589	Nucleoside-triphosphatase regulator activity	33	336	0.038
Biological Function	GO:0030695	GTPase regulator activity	30	296	0.0394
Biological Function	GO:0005244	Voltage-gated ion channel activity	23	194	0.0358
Biological Function	GO:0022843	Voltage-gated cation channel activity	21	137	0.0074
Biological Function	GO:0072509	Divalent inorganic cation transmembrane transporter activity	20	158	0.038
Biological Function	GO:0015085	Calcium ion transmembrane transporter activity	18	130	0.0336
Biological Function	GO:0005088	Ras guanyl-nucleotide exchange factor activity	17	109	0.0169
Biological Function	GO:0005262	Calcium channel activity	17	114	0.0242
Biological Function	GO:0005245	Voltage-gated calcium channel activity	11	44	0.0133
Biological Function	GO:0005089	Rho guanyl-nucleotide exchange factor activity	11	59	0.0443
Biological Function	GO:0045505	Dynein intermediate chain binding	10	30	0.0071
Biological Function	GO:0051959	Dynein light intermediate chain binding	8	29	0.038
Biological Function	GO:0008569	ATP-dependent microtubule motor activity, minus-end-directed	6	15	0.0394

The data is sorted based on the highest observed gene count.

our analysis encompasses various pathways, we acknowledge the central focus of our study on $CD4^+$ T cells and their role in the context of HIV-1 infection and ART treatment. We recognize the relevance of other studies that investigate the interaction of HIV-1 infection, ART, and lncRNAs in cells of the central nervous system, but for this chapter, we will concentrate on $CD4^+$ T cells and their potential effects on relevant biological processes in these cells.

Neurotoxicity in HIV-1-infected patients is believed to be mediated by HIV-1 proteins, such as gp120 and transactivator of transcription (Tat), as well as other products released by infected cells (Fitting et al., 2008). Our analysis of biological functions (BF) (Table 7) revealed enrichment in terms related to protein binding (*p-value* = 0.009), enzyme binding (*p-value* = 0.038), enzyme regulatory activity (*p-value* = 0.0191), and cytoskeletal protein binding (*p-value* = 0.0133). The role of cytoskeletal proteins, such as actin and profilin 1, has been implicated in the efficient cell-to-cell spread of HIV-1 (Kadiu et al., 2007; Taylor et al., 2011). Additionally, ion channel activity, including potassium channel activity and calcium channel activity, emerged as enriched BF terms. Previous studies have demonstrated the significance of ion channels in HIV-1 replication and gene expression (Choi et al., 1998; Ohno, 2018; Dubey et al., 2019).

Moreover, our analysis suggested a potential link between HIV-1 infection and early anatomical and functional changes in relevant brain regions, with implications for neuronal connectivity and glutamate release (Hu, 2016). We also

highlight a study indicating that Tat-mediated upregulation of a specific long non-coding RNA (lncRNA), LOC102549805 (lncRNA-U1), disrupts neuronal homeostasis, further supporting the notion that lncRNAs may play a role in regulating ion channels and cellular functions in the context of HIV-1 infection (Torkzaban et al., 2020).

In summary, our analysis, while encompassing various pathways and potential targets of lncRNAs, is centered on the context of $CD4^+$ T cells, HIV-1 infection, and ART treatment. Our findings suggest a complex interplay between lncRNAs, biological processes, and pathways that warrant further investigation to fully comprehend their mechanistic underpinnings.

Conclusion

In this study, we showed a thorough investigation into the role of lncRNAs in individuals infected with HIV-1 at various stages of infection and in response to ART. Our results produced a substantial set of 9,866 lncRNAs, separating 8,720 novel lncRNAs from 1,145 conserved counterparts. Particularly, we focused on the specific context of naïve $CD4^+$ T cells purified from PBMCs, contributing to a more defined characterization. Comparison with previous investigations uncovered variations in identified lncRNAs, attributable to thorough discovery methodologies and the special focus on naïve $CD4^+$ T cells. We detected characteristic expression patterns in patients receiving early and late ART, with a higher prevalence of downregulated lncRNAs. This aligns with earlier observations and could be attributed to the effect of prolonged ART and the cell-type specificity of lncRNA expression. Besides, two prominent lncRNAs, hu-lnc5646 and hu-lnc8565, were consistently upregulated in both early and late ART groups, warranting additional analysis as potential biomarkers for HIV-1 infection. Furthermore, the complicated interaction networks between identified lncRNAs and specific miRNAs underscored their regulatory potential, specifically in the context of ART. Our subcellular localization analysis emphasized a significant proportion of lncRNAs in the nucleus, proposing their involvement in epigenetic and transcriptional regulation. Notably, the differential localization across treatments implies complicated functions manipulated by the subcellular microenvironment. The cis-regulatory analysis uncovered potential targets of these lncRNAs, indicating their known roles in crucial biological functions.

The identification of pathways associated with axon guidance, PI3K-Akt signaling, and MAPK signaling in our work suggests possible links to neurological symptoms in HIV-1 patients. The results of our study provide insight into the potential role of lncRNAs in regulating ion channel activity, which might have significant implications for cellular function. The recognition of KEGG pathways, such as the glutamatergic synapse, highlights the crucial involvement of $CD4^+$ T cells in synaptic plasticity, which has significance for HIV-1-related neurocognitive diseases. Significantly, our research offers a thorough summary of the regulatory network that involves lncRNAs in the context of HIV-1 infection and antiretroviral therapy (ART). Although we have seen connections between identified lncRNAs and certain pathways, it is important to use caution since our work does not show direct causality or interactions. The identified lncRNAs, their interactions with miRNAs, and their localization offer constructive insights into their possible roles in modulating immune responses and cancer-related pathways. Future research endeavors should plan to reveal the detailed mechanisms by which lncRNAs function in different subcellular compartments and their effect on disease progression and treatment outcomes.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

NF and AG design and analysis of data. TK and NM-D wrote the draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/av.2024.11572/full#supplementary-material

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