Restored lncRNAs After ART Therapy Have Potential Key Roles in the Recovery From AIDS

Yingying Zhou
Zhongnan Hospital, Wuhan University
Yuqing Huang
Modern Virology Research Center, College of Life Sciences, Wuhan University
Tielong Chen
Zhongnan Hospital, Wuhan University
Wenjia Hu
Zhongnan Hospital, Wuhan University
Xiaoping Chen
Zhongnan Hospital, Wuhan University
Wei Hou
State Key Laboratory of Virology/Institute of Medical Virology/Hubei Province Key Laboratory of Allergy & Immunology, School of Basic Medical Sciences, Wuhan University
Qi Zhang
Modern Virology Research Center, College of Life Sciences, Wuhan University
Yong Xiong (yongxiong64@163.com)
Zhongnan Hospital, Wuhan University

Research Article

Keywords: lncRNA, RNA-seq, HIV-1

Posted Date: September 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-871811/v1

Abstract

Background: Many studies have shown that long noncoding RNAs (lncRNAs) derived from the host and human immunodeficiency virus (HIV) itself play important roles in virus-host interactions and viral pathogenesis. To identify potential key lncRNAs in the regulation of HIV pathogenesis, transcriptome analysis of peripheral blood mononuclear cells (PBMCs), which were derived from 6 HIV/acquired immunodeficiency syndrome (AIDS) subjects pre-HAART and post-HAART with effective control of plasma viremia (<20 HIV RNA copies/ml) and 6 healthy subjects, was performed by RNA sequencing (RNA-seq).

Results: We identified a total of 974 lncRNAs whose expression levels were restored to normal after ART therapy. The results of the cis-acting analysis showed that only six lncRNAs have cis-regulated target genes, among which the target gene RP11-290F5.1, interferon regulatory factors 2 (IRF2), could promote HIV replication. We also identified lncRNA CTB-119C2.1, which regulates most mRNAs with differential expression between pre- and post-HAART, and the differences were significant. We selected lncRNA CTB-119C2.1 for qRT–PCR verification, and the results were consistent with those of RNA-seq. RAB3A and GADD45A, two of the lncRNA CTB-119C2.1-associated genes, have been shown to be associated with HIV infection. KEGG analysis of lncRNA CTB-119C2.1-associated genes revealed that most of the genes are involved in the p53 signaling pathway or pathways related to cell circulation and DNA replication

Conclusion: In this study, we used RNA-seq to systematically compare the expression profiles of lncRNAs in HIV subjects between untreated and treated time points. We successfully identified some lncRNAs with differential expression during certain periods (no HIV infection, HIV infection before treatment, and after treatment). Their expression is associated with viral loads, and some of their regulating genes were found to be involved in HIV pathogenesis through bioinformatic analysis. These findings could help to reveal the underlying molecular mechanism of the progression of AIDS.

Introduction

Acquired immunodeficiency syndrome (AIDS) is a disease caused by HIV infection. According to the World Health Organization, approximately 37.9 million people around the world were infected with HIV by the end of 2018, and AIDS remains one of the biggest threats to global health.

HIV has the life cycle of the retrovirus, which is marked by the single-stranded RNA genome, which replicates through reverse transcription and can integrate into the host genome[1]. The effective replication of HIV requires host cell activation, which leads to increased efficiency in reverse transcription, integration, and viral gene expression, a result of the stimulation of host factors involved in HIV replication[2]. These host factors include cell receptors for viral infection, the proteins responsible for the integration of the viral genome and the maturation of viral particles. The main treatment for AIDS now focuses on antiretroviral therapy. Combination antiretroviral therapy (cART) has significantly reduced AIDS-related morbidity and mortality[3]. Although existing therapies have lengthened the lives of patients, they do not lead to a cure for AIDS or a complete elimination of the virus[4]. During an acute infection, CD4 + T cell depletion is caused by HIV-induced cell lysis, CD8 + T cell killing of infected cells, or cell apoptosis. During the chronic period, general immune activation and the gradual loss of new T cell production lead to a gradual decline in the number of CD4 + T cells[5]. A main reason why AIDS is so difficult to cure is the latent infection of HIV[6]. During HIV infection, the viral genome

integrates into the host CD4 + T cell genome and enters a transcriptional silencing state in the cell subpopulation, known as the incubation period of the virus[7]. Targeting CD4 + T cells during the incubation period of HIV remains a challenge to current treatment strategies for AIDS.

Long noncoding RNA (lncRNA) is a general term for noncoding RNA whose transcriptional length is greater than 200 nucleic acids[8]. They are mainly distributed in the nucleus and cytoplasm with tissue specificity. These lncRNAs usually do not encode proteins. They participate in a variety of biological processes, such as cell differentiation, canceration, and immune response, and play an important role in the occurrence and prevention of certain diseases[9]. LncRNAs regulate gene expression, chromatin organization, cell transport, RNA decay, and protein translation and affect protein second-generation localization, function, decay and turnover[10]. Studies have shown that after viral infection, intracellular lncRNAs can regulate viral replication or cellular metabolism and the immune response[11]. In the study of HIV infection, lncRNAs can control the number of in vivo CD4 + T cells, the apoptosis of infected cells, the life cycle of the virus, and the incubation period of the virus[7, 12]. The development of targeted molecular therapies that target lncRNAs involved in viral replication and pathogenesis may be of great significance to the treatment of AIDS[13, 14]. There are still great opportunities and challenges regarding the role of lncRNAs in AIDS progression, which need more comprehensive and in-depth studies.

In the treatment of HIV patients, despite the use of cART, patients continue to experience chronic immune activation and inflammation, which may lead to an increased risk of non-AIDS comorbidities, such as metabolic syndrome and cardiovascular disease[15]. Although viral replication is suppressed after the use of cART, the biomarkers of immune activation, inflammation, and clotting disorders do not fully return to normal, and sustained immune activation is a leading cause of non-HIV complications[16]. At present, there is no effective method of controlling HIV viral loads and simultaneously improving prognosis after antiviral therapy. The accumulating results of lncRNA research in HIV infection suggest a potential role of lncRNAs in clinical HIV treatment. In this study, we identified a few lncRNAs involved in HIV replication and pathogenesis. These findings could help to explain the molecular mechanism of the progression of AIDS and provide potential key molecules for targeted molecular therapy in the treatment of AIDS.

Material And Methods

Patients

We collected six HIV-infected patients who were treated and followed up at the AIDS Center of Zhong Nan Hospital of Wuhan University from December 2018 to December 2019. HIV/AIDS Diagnostic Criteria Reference to the Guidelines for the Diagnosis and Treatment of AIDS, 3rd Edition (2015 Edition). Additionally, the data of six healthy people who underwent physical examination in the physical examination center of Zhong Nan Hospital of Wuhan University during the same period were collected. The initial screening of HIV antibody was negative, and all routine indicators were normal.

All the study subjects were 18 years or older. The subjects were divided into two groups: the health group (6 persons) and the treatment group (6 persons included pre- and posttreatment data). All the cases in this study were from outpatients and inpatients of the Department of Infectious Diseases, Central South Hospital of Wuhan University, and no recent virus infection was found. Peripheral blood monocytes were separated by

density gradient centrifugation. They were cryopreserved in fetal calf serum (Gibco) containing 10% DMSO (Sigma) and stored in liquid nitrogen.

The study scheme was approved by the Ethics Committee of Zhong Nan Hospital of Wuhan University, and all participants signed the relevant informed consent form.

Laboratory testing

A total of 18 whole blood samples from 12 participants were collected. For flow cytometric analysis, whole blood was collected in k3 EDTA vials. Whole blood was permeabilized and fixed using Cytofix/Cytoperm (BD Pharmingen, San Jose, CA, USA) according to the manufacturer's protocol followed by staining for 20 min at room temperature in the dark with a cocktail of antibodies, including anti-CD4-APC, anti-CD8-PE, and anti-CD3-PerCP (BD Pharmingen, CA, USA). After staining, RBCs were lysed using BD FACS lysing solution (BD Pharmingen, San Jose, CA, USA) according to the manufacturer's instructions. More than 50,000 cells were acquired for flow cytometric analyses on a BD FACSCaliber, and the results were analyzed using TreeStar FlowJo software version 8.8.7.

HIV viral load was determined using NucliSens Easy Q HIV-1 v2.0 (bioMerieux, lyon, France), with a limit of detection of 20 copies/ml.

RNA isolation and quality control

Total RNA was isolated from PBMCs of 18 whole blood samples from 12 participants (5 ml each) using TRIzol reagent (Life Technologies). Total RNA was extracted using the Qiagen RNeasy kit (QIAGEN), and during the purification process of the sample, DNA enzyme (QIAGEN) was used for on-column digestion of the DNA. RNA quality and concentration were measured using an Agilent 2100 biological analyzer.

RNA sequencing

Ribosomal RNA molecules (rRNA) were eliminated using Ribo-Zero rRNA removal kits (Illumina). RNA sequencing libraries were constructed using the TruSeq Stranded Total RNA Library Prep Kit (Illumina) according to the manufacturer's instructions, and purified library products were evaluated using 2200 Tapestation (Agilent Technologies) and Qubit®2.0 (Thermo Fisher Scientific). Finally, single-stranded DNA molecules were clustered and sequenced for 150 cycles on an Illumina HiSeq 3000 system (Illumina).

Data clean process

The raw reads were trimmed of low-quality bases using a FASTX-Toolkit (v.0.0.13; http://hannonlab.cshl.edu/fastx_toolkit/). Then, the clean reads were evaluated using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc).

Read alignment and differentially expressed gene (DEG) analysis

Clean reads were aligned to the human GRch38 genome by tophat2[17], allowing 4 mismatches. Uniquely mapped reads were used to calculate read number and reads per kilobase of exon per million fragments mapped (RPKM) for each gene. The expression levels of genes were evaluated using RPKM. The software edgeR[18], which is specifically used to analyze the differential expression of genes, was applied to screen the

RNA-seq data for DEGs. The results were analyzed based on the fold change (FC ≥ 2 or ≤ 0.5) and p value (p value ≤ 0.05) to determine whether a gene was differentially expressed.

LncRNA prediction and direction identification

To systematically analyze the lncRNA expression pattern, we used a pipeline for lncRNA identification similar to that previously reported[19], which was constructed based on cufflinks software[20]. All steps of the pipeline have been shown in Fig. 1A.

Coexpression analysis

To explore the regulatory mode between lncRNAs and mRNAs, we calculated the Pearson's correlation coefficients (PCCs) between them with a correlation of 0.6 and a p value of 0.05. Then, the distance between lncRNAs and genes (10 kb) was used as the threshold to determine the cis- and trans-regulatory relationships. A distance between lncRNAs and genes less than 10 kb was defined as cis regulation, and a distance greater than 10 kb or on other chromosomes was considered trans regulation.

Predicting Targets of trans- and cis-Acting lncRNA

We first identified all the coexpression pairs of lncRNAs and genes in each sample by calculating Pearson's correlation coefficients (|r| >0.6 and P value < 0.05) between the expression levels of differential lncRNAs and all genes. Then, with a threshold distance of 10 kb between lncRNA and gene pairs, cis-acting genes were identified. The other lncRNA and gene pairs with distances greater than 10 kb or in the different chromosomes were regarded as trans-acting pairs.

Functional enrichment analysis

To sort out functional categories of genes, Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were identified using the KOBAS 2.0 server[21]. Hypergeometric tests and Benjamini-Hochberg FDR controlling procedures were used to define the enrichment of each term. Reactome (http://reactome.org) pathway profiling was also used for functional enrichment analysis of the sets of selected genes.

Real-time quantitative PCR validation

A total of 16 whole blood samples (5 ml per piece) were collected from 8 participants, and PBMCs were isolated. Total RNA was extracted using the RNeasy Mini Kit (Qiagen). The RNAs were reverse transcribed with a reverse transcription kit (riboSCRIPTTM mRNA/lncRNA qRT–PCR Starter Kit (20T RT + 60T PCR) (Guangzhou RuiboBio Co., Ltd.). Applied Biosystems™ SYBR™ Green master mixes (Thermo Fisher Scientific) were used for RT–qPCR. GADPH serves as an internal reference gene. The relative expression of lncRNAs was calculated by the $2^{-\triangle\triangle Ct}$ method. Statistical analysis of qRT–PCR was performed by GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA), and comparison between the two samples was performed by t-test.

Other statistical analysis

Principal component analysis (PCA) analysis was performed by the R package factoextra (https://cloud.r-project.org/package=factoextra) to show the clustering of samples with the first two components. After normalizing the reads by FPKM (fragments per kilobase of exon model per million mapped fragments) of each

gene in samples. The pheatmap package (https://cran.r-project.org/web/packages/pheatmap/index.html) in R was used to perform clustering based on Euclidean distance. Student's t-test was used for comparisons between two groups. Cytoscape (v3.5.1) was used to display the network of lncRNAs and mRNAs. PPI analysis was performed by an online website (https://string-db.org/).

Results

Clinical characteristics of the volunteers

In the healthy control group, six patients undergoing physical examination in the Physical Examination Center of Zhong Nan Hospital were labeled H1, H2, H3, H4, H5 and H6. The treatment group consisted of 6 HIV-infected patients who were treated in the AIDS Center of Zhong Nan Hospital of Wuhan University and labeled B1, B2, B3, B4, B5, and B6 before treatment or A1, A2, A3, A4, A5, and A6 after treatment. Each participant's age, sex, CD4 + and CD8 + T cell counts, plasma viral load, and HAART regimen are shown in Table 1. After HAART treatment, all 6 HIV-infected subjects' viral loads were below the threshold of detection.

Table 1

Subjects' age, sex, CD4+ T lymphocyte count, CD8+ T lymphocyte count, plasma viral load, and HAART regimen.

Label	Gender	Age	Detection time	CD4+T lymphocyte (count/ul)	CD8+T lymphocyte (count/ul)	Plasma viral load (copies/ml)	cART regiment
H1	Female	24	2019/12/17				
H2	Female	25	2019/12/17				
H3	Female	25	2019/12/17				
H4	Male	20	2019/12/17				
H5	Female	25	2019/12/17				
H6	Female	24	2019/12/17				
B1	Male	30	2019/4/2	61	699	13799	TDF+3TC+EFV
A1	Male	-	2019/9/29	112	900	<20	
B2	Male	29	2019/4/17	236	1106	26853	TDF+3TC+EFV
A2	Male		2019/7/15	321	666	<20	
B3	Male	27	2019/4/19	266	261	96164	TDF+3TC+EFV
A3	Male		2019/10/15	366	550	<20	
B4	Male	31	2019/4/29	393	504	4704	TDF+3TC+EFV
A4	Male		2019/10/28	648	660	<20	
B5	Male	19	2019/4/29	400	590	5974	TDF+3TC+EFV
A5	Male		2019/8/15	642	763	<20	
B6	Male	48	2019/5/16	283	519	38386	TDF+3TC+EFV
A6	Male		2019/8/14	310	255	<20	

Identification of differentially expressed lncRNAs between the HAART treatment group and the healthy group

RNA-seq was performed on the PBMCs of subjects from the healthy control group or treatment group (each subject's PBMCs were collected before and after treatment in pairs). The genes and lncRNAs responding to HIV infection and HAART treatment were identified by pairwise comparison among the three groups, and then the functions of the lncRNAs and their regulatory genes were further determined (Fig. 1A). After RNA-seq, principal component analysis (PCA) was performed on the normalized expression levels of all the expressed genes.

There was a clear distinction between the healthy and treatment groups (both before and after treatment) (Fig. 1B). Then, we pairwise compared the three groups of samples and obtained the genes with significantly upregulated expression or significantly downregulated expression after HIV infection (before vs. healthy, Fig. 1C). Patients treated with HAART showed a decrease in the number of both upregulated and downregulated genes, which means that the abnormally expressed genes gradually restored to a normal expression pattern after treatment (comparison between before vs. healthy and after vs. healthy). The results showed that 2759 genes were upregulated after HIV infection, among which 198 genes were restored to normal expression and 67 genes were decreased but still higher than those of healthy controls after HAART treatment. Additionally, 4,545 genes expressions were downregulated after HIV infection, among which 421 genes being restored to normal expression and 117 genes expressions were upregulated but still lower than those of healthy controls after HAART treatment (Fig. 1D). Most of the gene expression levels were restored to normal after treatment (Fig. 1E).

Gene Ontology biological process

In HIV patients, most upregulated genes were involved in biological processes related to inflammation/immune response, while most downregulated genes were involved in biological processes related to cell-cell and cell-matrix. Analysis showed that some of the upregulated gene expression and inflammation levels decreased after treatment; however, they were still abnormal. (Fig. 2).

Features of lncRNAs expression profiling

We further identified and classified lncRNAs (Fig. 3A). A total of 6046 lncRNAs with differential expression, including genome-annotated and predicted lncRNAs, were identified in the three groups of samples (the healthy people, pretreatment, and posttreatment groups) (Fig. 3B). The results of principal component analysis (PCA) of all lncRNA expression showed that the healthy group and the disease group were distinct from each other. The results of the PCA of differentially expressed lncRNAs and differentially expressed genes indicated that the difference in lncRNA expression was more obvious than that in gene expression (Fig. 3C). This suggests that lncRNAs may play a significant role in AIDS. Then, we analyzed the lncRNAs whose expression levels were significantly upregulated or downregulated during HIV infection or treatment (Fig. 3D). Among all identified lncRNAs, 268 abnormally upregulated lncRNAs were restored to normal levels after HAART treatment, while 82 abnormally upregulated lncRNAs were decreased but remained higher than those of the healthy group. Correspondingly, 706 abnormally downregulated lncRNAs restored to normal levels after HAART treatment, while 200 abnormally downregulated lncRNAs increased but remained lower than those of the healthy group (Fig. 3E). Finally, we found that a total of 974 lncRNAs showed a tendency to recover to the level of healthy people after treatment (Fig. 3F).

Exploration of the potential roles of lncRNAs through trans- and cis-acting analysis

We analyzed the target genes of 974 lncRNAs, whose expression was restored to that of healthy people after HAART treatment. First, we investigated the expression relationship between the restored lncRNAs and their associated genes (Fig. 4A). According to the cis-regulation analysis, six lncRNAs with homeopathic regulation target genes were identified, one of which is lncRNA RP11–290F5.1, whose target gene IRF2 was reported to be related to HIV infection (Fig. 4B). Then, we analyzed the differential expression of lncRNAs before and after HAART treatment by coexpression analysis (Fig. 4C). We identified that lncRNA CTB-119C2.1, with the most

obvious expression difference, was the lncRNA with the largest number of associated differentially expressed genes. Based on the results of coexpression, GO enrichment analysis was performed on these genes (Fig. 4D). The top seven biological processes in which these genes are enriched are most likely to be involved in a series of complications caused by HIV-1 infection. The regulatory network among differentially expressed lncRNAs, coexpressed genes, and associated biological processes during HIV infection and treatment was constructed (Fig. 4E). Among the lncRNA-associated genes, IL-1α, IL-10, CCL2, LMNA and VCAM1 were associated with HIV infection (Fig. 4F).

Real-time quantitative PCR validation

The lncRNAs mentioned above, which were predicted to play important roles during HIV infection, were validated by RT–qPCR. We selected lncRNA CTB-119C2.1 with the most significant expression difference and the largest number of coexpressed genes for qRT–PCR verification, and the results showed trends consistent with the RNA-seq results (Fig. 5A). Then, the coexpressed genes of lncRNA CTB-119C2.1 were analyzed to reveal the potential function of this lncRNA (Fig. 5B). KEGG analysis of the associated genes showed that genes were most enriched in p53 signaling. among which RAB3A and GADD45A have been shown to be associated with HIV infection (Fig. 5C).

Discussion

In this study, we compared the expression profiles of lncRNAs before and after AIDS treatment and further analyzed their potential function. A total of 974 lncRNAs and 619 associated genes, whose expression levels became abnormal after HIV infection and restored to normal after treatment, were identified. We believe that these lncRNAs, along with their associated genes, may have a potential impact on HIV replication.

LncRNAs have many functions in vivo, participating in the epigenetic, transcriptional, posttranscriptional, and translational regulation of genes and acting as vectors in biological processes[22]. Studies have found that the expression or functional abnormalities of lncRNAs are closely related to the occurrence of human diseases, including cancer[23, 24], neurodegenerative diseases[10] and diabetes[25]. LncRNAs also play an important role in viral infection, and some studies have shown that lncRNAs are involved in the regulation of many biological processes during HIV infection[26]. RNA-seq is a quantitative and extremely sensitive technique for genome-wide transcriptome analysis based on sequencing. This technique and its derivative methods have been widely used to identify potential lncRNAs in several species[27]. In this study, we analyzed the expression changes of lncRNAs in PBMCs of healthy people and HIV-infected patients before and after HAART treatment by RNA-seq.

Many genes showed significant abnormal expression patterns during HIV infection, and some of these genes were restored to normal expression after treatment. The upregulated genes were enriched in immune/inflammatory processes, while the downregulated genes were enriched in cell-cell/cell-matrix processes. Although the expression of inflammation-related genes decreased after treatment, there was still widespread inflammation in the infected patients. The expression levels of genes in cell-cell/cell-matrix processes decreased after treatment, which may be related to the persistence of the consequences of HIV-1 infection.

The PCA clustering results of identified lncRNAs showed significant expression differences during HIV infection and treatment. We found that a total of 974 abnormally expressed lncRNAs (268 upregulated lncRNAs and 706

downregulated lncRNAs) were restored to normal expression levels after treatment. Homeopathic regulation target gene analysis of the lncRNAs showed that RP11–685M7.3, AC002550.5, RP 11–290F5.1, RP 11–96D1.3, RP 5–1057120.4, and RP 11–972P1.10 have cis-regulation target genes. These lncRNAs may have a potential role in HIV infection and pathogenesis. For example, interferon regulatory factor 2 (IRF2), a cis-regulatory target gene of lncRNA RP 11–290F5.1, has been reported to be associated with HIV infection[28]. As the most representative members of the IRF family, IRF1 and IRF2 can be involved in a variety of biological processes, including inflammation, immune response, cell proliferation, and differentiation[29]. Previous studies have shown that a sequence homologous to ISRE, which is the binding site of IRF1 and IRF2, is in the 5' LTR downstream of HIV-1. Deletion of the LTR-ISRE sequence leads to impaired LTR promoter activity and decreased synthesis of viral RNA and proteins[30]. In the absence of viral transactivator (Tat), IRF2 can bind to the LTR-ISRE sequence and drive LTR transcription[31]. The IRF2 gene is a cis-regulatory target of lncRNA RP11–290F5.1, suggesting that this lncRNA may play a regulatory role in the process of HIV-1 infection.

By coexpression analysis of differentially expressed lncRNAs and genes, transregulatory targets of lncRNAs were identified. The results indicated that the target genes participate in multiple biological processes, including the immune inflammatory response, angiogenesis, cell proliferation, and mitotic cell cycle, and these genes could be involved in the complications caused by HIV-1 infection[32–35]. For example, IL-1α, IL-10, CCL2, LMNA and VCAM1, the target genes identified in this study, have been reported to be associated with HIV-1 infection and pathogenesis[36–38]. It is worthwhile to further explore the regulatory relationship between the lncRNAs and their target genes identified in this study. The lncRNA CTB-119C2.1, with the most significant differential expression pattern and the most coexpression-associated genes, was selected for qRT–PCR verification. Its qRT–PCR results were consistent with those of RNA-sEq. The results indicated that the abnormally downregulated expression level of lncRNA CTB-119C2.1 during HIV infection increased after treatment. The following coexpression analysis showed that its associated genes were mainly involved in the p53 pathway and the signaling pathways responsible for the cell cycle and DNA replication, suggesting that lncRNA CTB-119C2.1 may play an important role during HIV infection and treatment. The detailed mechanism needs to be clarified in further studies[39–41].

There are also some limitations in this study. First, only total PBMCs were investigated in this study, and further studies are needed to clarify the role of lncRNAs in specific cell types. Second, although the regulatory network of lncRNA-related genes has been obtained, more experiments are still needed to confirm the results. Third, the number of patients investigated in this study may be small, and more samples should be included in the future to confirm the universality of the results.

Conclusion

In conclusion, our study proved that HIV infection can cause abnormal expression of lncRNAs and their associated genes, which can be restored to normal after effective HAART treatment. Bioinformatic analysis revealed that lncRNA RP11–290F5.1 and lncRNA CTB-119C2.1 may play a key role in HIV infection and pathogenesis, which is worthy of further exploration of the specific mechanism.

Abbreviations

LncRNAs: long noncoding RNAs

HIV: human immunodeficiency virus

PBMCs: peripheral blood mononuclear cells

IRF2: interferon regulatory factors 2

RNA-seq: RNA-sequencing

AIDS: acquired immunodeficiency syndrome

cART: combination antiretroviral therapy

RPKM: reads per kilobase of exon per million fragments mapped

PCCs: Pearson's correlation coefficients

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

PCA: Principal component analysis

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the Medical Ethics Committee, Zhongnan Hospital of Wuhan University. The reference number is 2019-119.

Consent to publication

All authors approved the manuscript and agreed to publish.

Availability of data and materials

All relevant data and materials during this study are included in this published article.

Competing interests

The authors declare no conflict of interest.

Funding

This study was supported by the Translational Medicine and Interdisciplinary Research Joint Fund of Zhongnan Hospital of Wuhan University (Grant No. ZNLH201905), the Medical Science Advancement Program (Basical Medical Sciences) of Wuhan University (Grant No. TFJC2018002), and the National Key Research and Development Program of China (2018YFE0204503).

Contributions

YZ and YX designed the whole experiment, participated in the data collection and analysis, and were major contributors to drafting the manuscript. TC, WH and XC contributed to data collection and the implementation of the experiment. YH was responsible for the analysis and manuscript drafting. WH and QZ modified the manuscript. All authors read and approved the final manuscript.

YZ and YH contributed equally to this work.

Acknowledgments

We would like to thank all staff in the Department of Infectious Diseases, Zhongnan Hospital of Wuhan University.

References

1. Freed, Eric OJNRM. HIV-1 assembly, release and maturation. 2015; 13(8): 484-96.
2. Ferguson MR, Rojo DR, Lindern J, O'Brien WA. Iconography : HIV-1 replication cycle.
3. Aids NJ. The effect of combined antiretroviral therapy on the overall mortality of HIV-infected individuals. 2010; 24(1): 123-37.
4. Lint CV, Bouchat S, Marcello AJR. HIV-1 transcription and latency: an update. 2013; 10(1): 67.
5. B ZHA, B MG, C JMBJTiI. HIV infection: first battle decides the war. 2006; 27(6): 274-81.
6. Finzi D, Blankson J, Siliciano JD, Margolick JB, Siliciano RFJNM. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. 1999; 5(5): 512.
7. Saayman S, Roberts TC, Morris KV, Weinberg MSJOTtTX. HIV Latency and the noncoding RNA therapeutic landscape. 2015; 848: 169-89.
8. Mercer, Tim, R., et al. Long non-coding RNAs: insights into functions. 2009.
9. Lee C, Kikyo NJCB. Strategies to identify long noncoding RNAs involved in gene regulation. 2012; 2.
10. Knauss JL, Sun TJN. Regulatory mechanisms of long noncoding RNAs in vertebrate central nervous system development and function. 2013; 235: 200-14.
11. Peng X, Gralinski L, Armour CD, Ferris MT, Katze MGJM. Unique Signatures of Long Noncoding RNA Expression in Response to Virus Infection and Altered Innate Immune Signaling. 2010; 1(5).
12. Ray RM, Morris KV. Long Non-coding RNAs Mechanisms of Action in HIV-1 Modulation and the Identification of Novel Therapeutic Targets. 2020.
13. Boliar S, Gludish DW, Jambo KC, Kamng'Ona R, Russell DGJPotNAoS. Inhibition of the lncRNA SAF drives activation of apoptotic effector caspases in HIV-1–infected human macrophages. 2019; 116(15): 201818662.
14. Li H, Chi X, Li R, Ouyang J, Chen YJMT-NA. A Novel lncRNA, AK130181, Contributes to HIV-1 Latency by Regulating Viral Promoter-Driven Gene Expression in Primary CD4+ T Cells. 2020; 20.

15. Zicari S, Sessa L, Cotugno N, et al. Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. 2019; 11(3).
16. Hsu DC, Sereti IJD. Serious Non-AIDS Events: Therapeutic Targets of Immune Activation and Chronic Inflammation in HIV Infection. 2016; 76(5): 533-49.
17. Kim D, Pertea G, Trapnell C, Pimentel H, Biology RKJG. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. 2013; 14(4).
18. Robinson M, Mccarthy DJ, Smyth GKJB-O-. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. 2010.
19. Liu S, Wang Z, Chen D, et al. Annotation and cluster analysis of spatiotemporal- and sex-related lncRNA expression in rhesus macaque brain. 2017; 27(9): 1608-20.
20. Trapnell C RA, Goff L, Pertea G, Kim D, Kelley DR %J Nature Protocols. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. 2012; 7: 562-78.
21. Chen X, Mao X, Huang J, et al. KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. 2011; 39(Web Server issue): 316-22.
22. Khorkova O, Hsiao J, Wahlestedt CJADDR. Basic biology and therapeutic implications of lncRNA. 2015; 87: 15-24.
23. Peng WX, Koirala P, Mo YYJO. LncRNA-mediated regulation of cell signaling in cancer. 2017.
24. Yang G, Lu X, Yuan LJBEBA. LncRNA: a link between RNA and cancer. 2014; 1839(11): 1097-109.
25. Liu JY, Yao J, Li XM, Song YC, Jiang QJCD, Disease. Pathogenic role of lncRNA-MALAT1 in endothelial cell dysfunction in diabetes mellitus. 2014; 5(10): e1506.
26. Li SA, Cw B, Jz A, et al. Roles and potential applications of lncRNAs in HIV infection - ScienceDirect. 2020; 92: 97-104.
27. Jathar S, Kumar V, Srivastava J, Tripathi V. Technological Developments in lncRNA Biology: Long Non Coding RNA Biology, 2017.
28. Simmons A, Aluvihare V, Mcmichael AJI. Nef Triggers a Transcriptional Program in T Cells Imitating Single-Signal T Cell Activation and Inducing HIV Virulence Mediators. 2001; 14(6): 763-77.
29. Taniguchi T, Ogasawara K, Takaoka AA, Tanaka NJARoI. IRF family of transcription factors as regulators of host defense. 2001; 19(1): 623.
30. Battistini A, Marsili G, Sgarbanti M, Ensoli B, Hiscott JJJoI, Research C. IRF regulation of HIV-1 long terminal repeat activity. 2004; 22(1): 27-37.
31. Marsili G, Remoli AL, Sgarbanti M, et al. HIV-1, interferon and the interferon regulatory factor system: An interplay between induction, antiviral responses and viral evasion. 2012; 23(4-5): 255-70.
32. Kuller LH, Russell T, Waldo B, et al. Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection. 2008; 5(10): e203.
33. Albini A, Soldi R, Giunciuclio D, et al. The angiogenesis induced by HIV|[ndash]|1 Tat protein is mediated by the Flk|[ndash]|1/KDR receptor on vascular endothelial cells. 1996; 2(12): 1371-5.
34. Reeves DB, Duke ER, Wagner TA, Palmer SE, Spivak AM, Schiffer JTJNC. A majority of HIV persistence during antiretroviral therapy is due to infected cell proliferation.

35. K., Sakai, J., et al. The Vif and Vpr accessory proteins independently cause HIV-1-induced T cell cytopathicity and cell cycle arrest. 2006; 103(9): 3369-74.
36. Roberts L, Passmore J, Williamson C, et al. Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. 2010; 24(6): 819-31.
37. Caron M, Auclair M, Donadille B, et al. Human lipodystrophies linked to mutations in A-type lamins and to HIV protease inhibitor therapy are both associated with prelamin A accumulation, oxidative stress and premature cellular senescence. 2007; 14(10): 1759-67.
38. Kai KL, Chi D, Li CC, Hall H, Milhorn D, Krishnaswamy GGJAJPLCMP. HIV-1 Tat protein-induced VCAM-1 expression in human pulmonary artery endothelial cells and its signaling. 2005; 289(2): 252-60.
39. Perfettini, Medicine J-LJJoE. Essential role of p53 phosphorylation by p38 MAPK in apoptosis induction by the HIV-1 envelope. 2005; 201(2): 279-89.
40. McCune, Joseph MJN. The dynamics of CD4+ T-cell depletion in HIV disease. 2001; 410(6831): 974-9.
41. Gianella S, Anderson C, Var SR, et al. Replication of Human Herpesviruses Is Associated with Higher HIV DNA Levels during Antiretroviral Therapy Started at Early Phases of HIV Infection. 2016: JVI.02638-15.

Figures

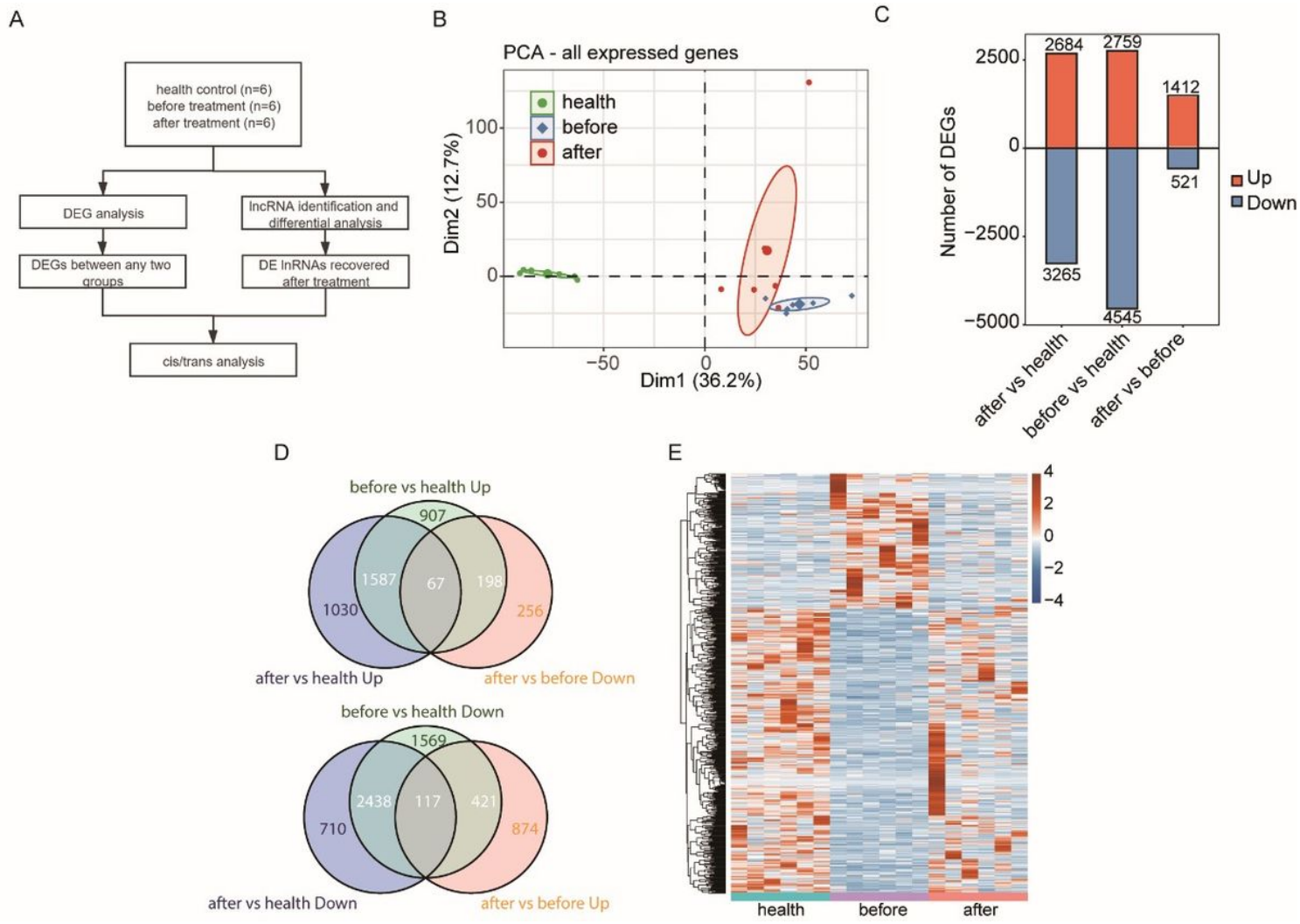

Figure 1

After treatment, the expression levels of many genes showed a recovering tendency to the level of healthy people. (A) Illustration of the experimental design and bioinformatics analysis pipeline for this study; (B) Principal component analysis (PCA) of samples from three groups based on the normalized expression level of all expressed genes. The samples were grouped by disease and treatment state, and the ellipse for each group is the confidence ellipse; (C) The number of DEGs among different groups. The numbers of upregulated and downregulated DE lncRNAs are shown in a bar plot. (D) Venn plot showing differentially expressed genes between treatment samples and healthy controls. samples, and between after- and before treatment. Up: upregulated; Down: downregulated; (E) Heatmap showing the expression profile of restored genes after treatment, which is 198 genes and 421 genes in (D).

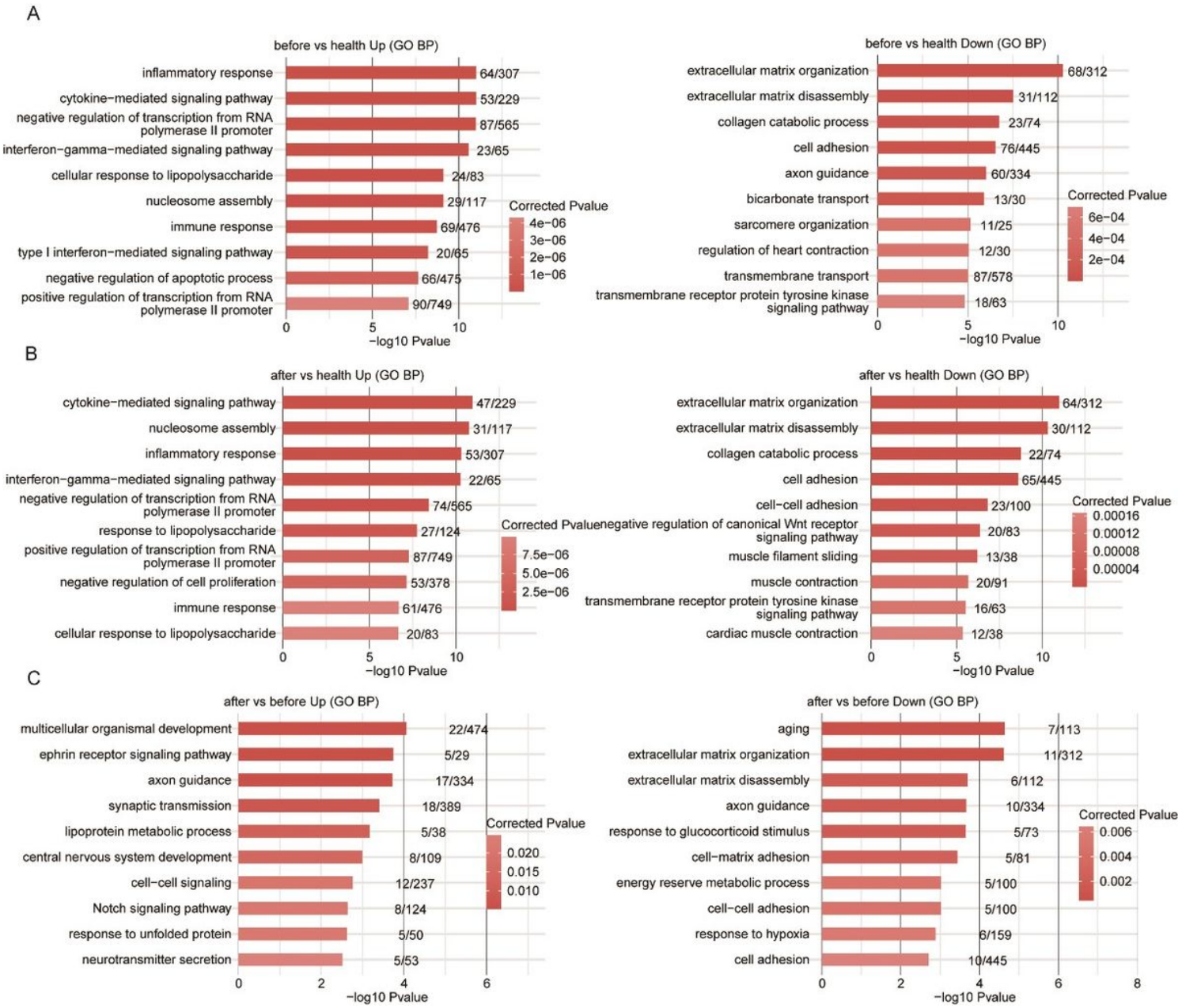

Figure 2

Patients still had extensive immune responses and persistent postinfection damage after treatment. (A) Top 10 GO biological process terms enriched by differentially expressed genes compared between the before and healthy groups. (B) Top 10 GO biological process terms enriched by differentially expressed genes compared between the after and healthy groups. (C) Top 10 GO biological process terms enriched by differentially expressed genes compared between the after and before groups.

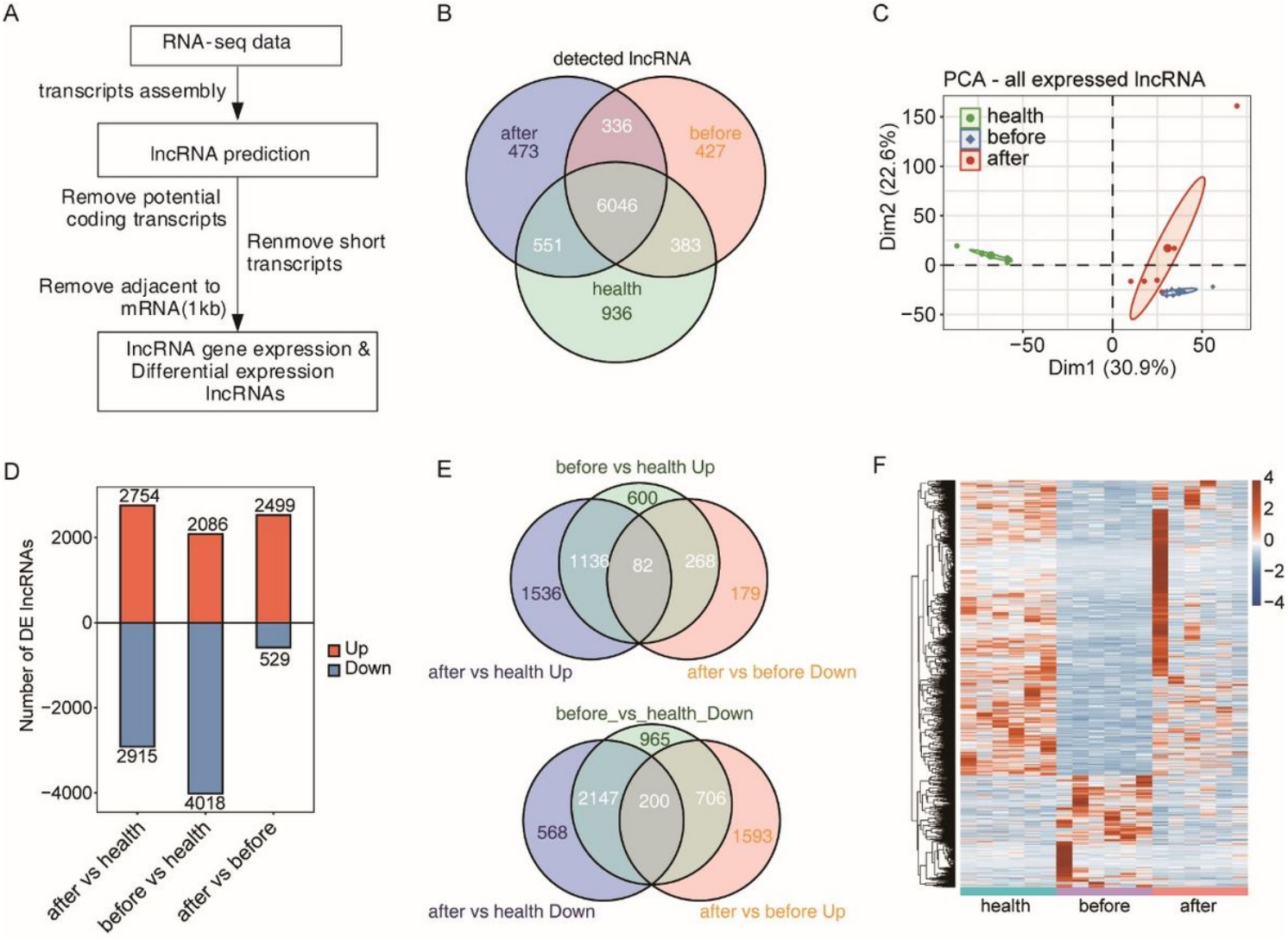

Figure 3

A comprehensive catalog of lncRNAs whose expression levels recovered to no significant difference from those of healthy controls after therapy. A) Illustration of the experimental design and bioinformatics analysis pipeline for the identification of lncRNA genes expressed in samples of three groups. (B) Venn diagram of detected lncRNAs in samples of three groups. The lncRNAs that were detected (RPKM ≥ 0.2) in at least two samples of any group were included in this analysis. (C) Principal component analysis (PCA) of samples from three groups based on the normalized expression level of all expressed lncRNAs. The samples were grouped by disease and treatment state, and the ellipse for each group is the confidence ellipse. (D) The number of DE lncRNAs among different groups. The number of upregulated and downregulated DE lncRNAs is shown in a bar plot. (E) Venn plot showing differentially expressed lncRNAs between treatment samples and healthy samples and between

after- and before treatment. Up: upregulated; Down: downregulated. (F) Heatmap showing the expression profile of restored lncRNAs after treatment, which is 268 genes and 706 genes in (E).

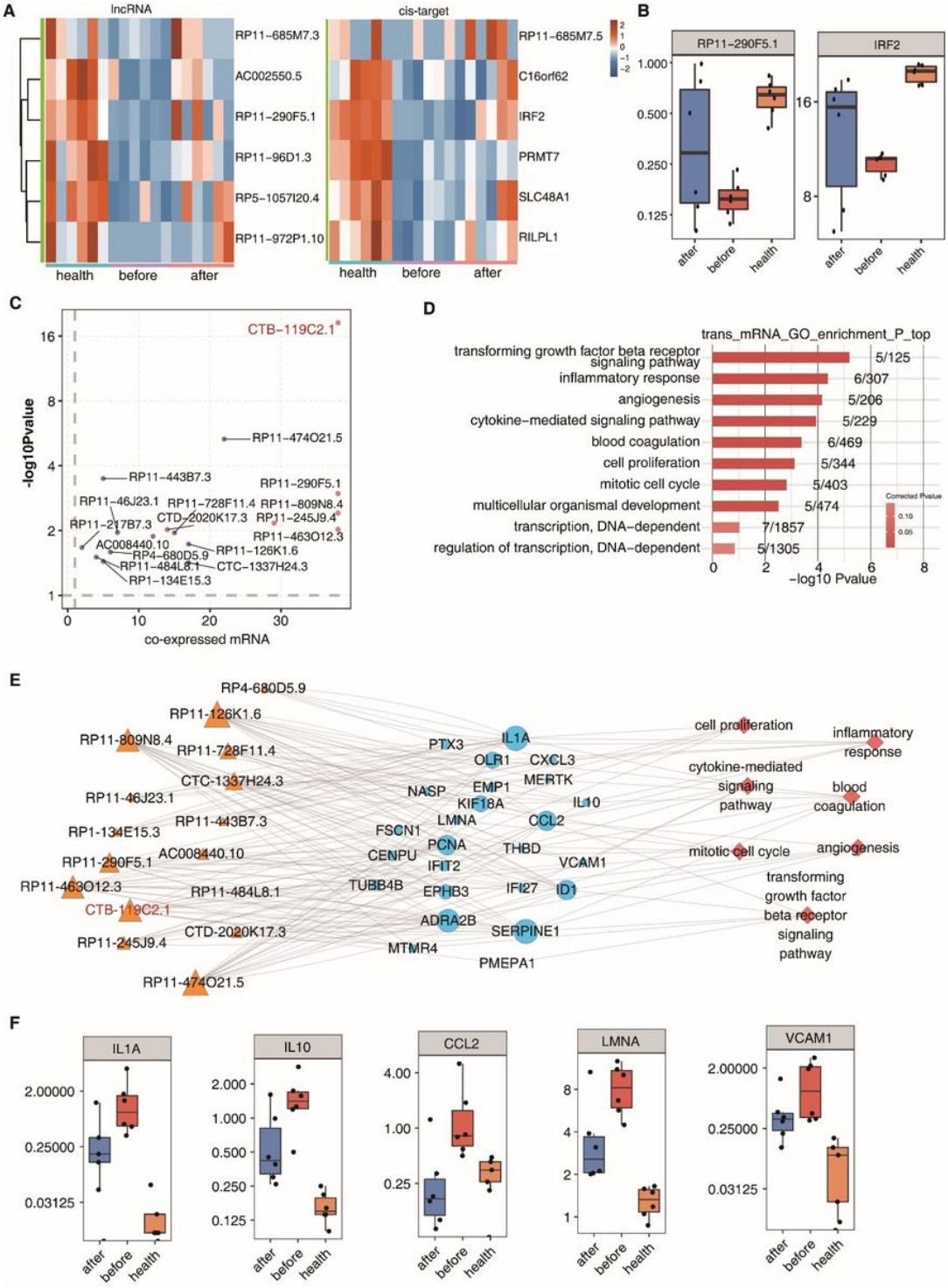

Figure 4

Trans- and cis-acting analysis of lncRNAs whose expression levels recovered to no significant difference with healthy people after therapy. (A) Heatmap showing the cis-acting pairs of restored lncRNAs after treatment (left) and target genes (right). (B) Boxplots showing the expression status of lncRNA RP11−290F5.1 and its cis-target IRF2 in the healthy, before, and after groups. (C) Scatter plot shows DE lncRNAs by after-treatment compared with before-treatment samples and the number of coexpressed DE genes. Red points denote

upregulated lncRNAs involved in coexpression pairs, and blue points denote downregulated lncRNAs. Cutoffs of p value < 0.01 and Pearson coefficient ≥ 0.6 were applied to identify the coexpression pairs. Low-abundance genes were filtered out when the expression of more than 20% of genes was less than 0.5. (D) GO biological process terms enriched by trans-target genes. (E) The coexpression network between DE lncRNAs and DE genes that are involved in the top 7 GO terms shown in (D). LncRNAs are on the left, coexpressed genes are in the center, and the gene-enriched GO terms are on the right. (F) Boxplots showing the expression status of five trans-target genes from (E) in the healthy, before, and after groups.

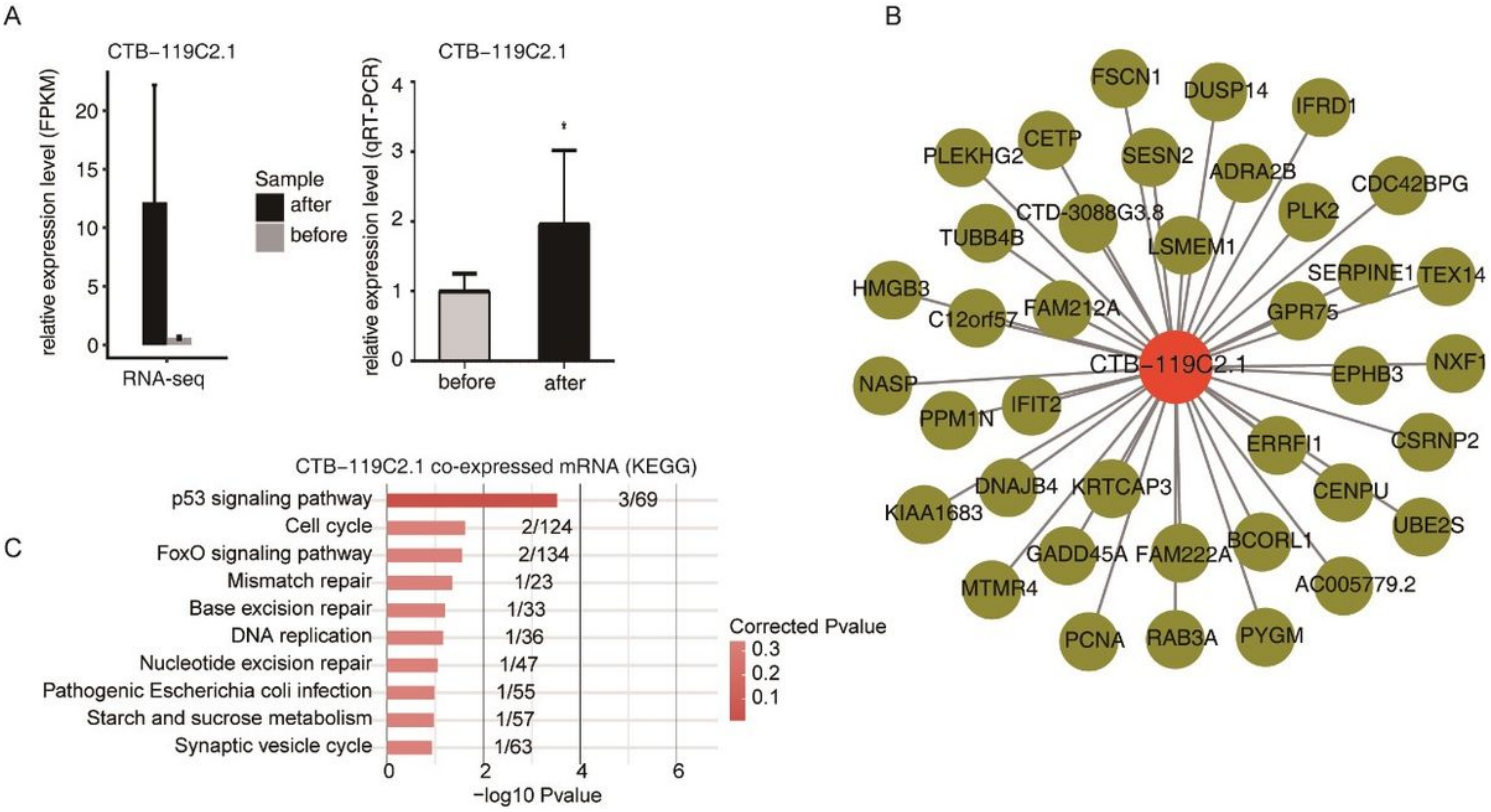

Figure 5

Validation of differential expression of lncRNA CTB-119C2.1 before and after samples and analysis of coexpressed genes. (A) Bar plots showing the relative expression level of CTB-119C2.1 using RNA-seq data (left) and qRT–PCR (right) in the before and after groups. (B) Coexpressed DE genes of CTB-119C2.1. (C) Bar plot showing the top 10 enriched KEGG pathways from coexpressed genes of CTB-119C2.1.