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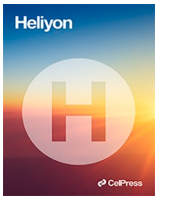

Research article

Dynamic transcriptome analyses reveal m^{6}A regulated immune non-coding RNAs during dengue disease progression

Ya Zhang [a,1], Jing Guo [a,1], Yueying Gao [a,1], Si Li [a], Tao Pan [a], Gang Xu [a], Xia Li [a,b,*], Yongsheng Li [a,**,2], Jun Yang [a,***]

[a] Key Laboratory of Tropical Translational Medicine of Ministry of Education, College of Biomedical Information and Engineering, Hainan Women and Children's Medical Center, Hainan Medical University, Haikou 571199, China
[b] College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150081, China

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ABSTRACT

Dengue infection is one of the most prevalent arthropod-borne viral diseases, which can result in severe complications. Identification of genes and long non-coding RNAs (lncRNAs) involved in dengue infection would help in deciphering potential mechanisms responsible for the disease progression. We comprehensively analyzed the dynamic transcriptome during dengue disease progression and identified critical genes and lncRNAs with expression perturbations. Our findings revealed that the expression of genes (i.e., CCR10 and GNG7) and lncRNAs (i.e., CTBP1-AS and MAFG-AS1) were potentially regulated by m6A RNA methylation. Interestingly, dengue viral proteins prevalently interact with genes or lncRNAs with expression perturbations, which are involved in cell cycle, inflammation signaling pathways and immune response. Dynamically expressed genes and lncRNAs were likely to locate in the central regions of human protein-protein network, which play crucial roles in mediating signaling spread and helping viral replication. Immune microenvironments analysis revealed that plasma cells levels were increased and T cells infiltrations were decreased during dengue disease progression. Dynamically expressed genes and lncRNAs were correlated with immune cell infiltrations. Moreover, network analysis reveals the associations between dengue viral infections and human complex diseases (i.e., digestive diseases and neoplasms). Our comprehensive transcriptome analysis of dengue disease progression identified potential gene and lncRNA biomarkers, providing novel insights for understanding the pathogenesis of and developing effective therapeutic strategies for dengue infection.

1. Introduction

Dengue fever is an acute insect-borne disease caused by mosquito-borne transmission of dengue virus. Dengue viruses (DENV) are

* Corresponding author.Key Laboratory of Tropical Translational Medicine of Ministry of Education, College of Biomedical Information and Engineering, Hainan Women and Children's Medical Center, Hainan Medical University, Haikou 571199, China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: lixia@hrbmu.edu.cn (X. Li), liyongsheng@hainmc.edu.cn (Y. Li), hy.yj0101@hainmc.edu.cn (J. Yang).
[1] These authors contributed equally to this work.
[2] Lead contact.

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members of the *Flaviviridae* family, which is widespread throughout the tropics [1]. It is subdivided into four serotypes, DENV1, DENV2, DENV3 and DENV4 [2,3]. First, dengue virus infects mosquitoes and then multiplies in their bodies for 8–12 days to infect humans. At the beginning of DENV infection, the human body shows symptoms similar to other febrile illnesses (OFI). At this stage, it is called the dengue infection without any warning sign (DI). Warning signs develop 3–7 days after the first symptoms (DWS) that include rapid breathing, bleeding gums, fatigue, restlessness, and severe abdominal pain. Untreated DWS will evolve into a potentially lethal complication called severe dengue (DS) leading to fluid accumulation, respiratory distress, and severe bleeding [4]. DENV interacts with multiple processes of host to facilitate their replication. Numbers of reports have extensively studied the interactions among DENV and host proteins [5,6], and host immunology factors and virus serotypes are known to modulate the DENV infection [7]. However, there are still certain underlying host factors that play fundamental roles in modulating DENV infection remains to be discovered.

In addition to protein-coding genes, the majority of human genome is transcribed into non-coding transcripts, which play critical roles in regulating the expression of genes [8]. Several reported studies have suggested that non-coding RNAs (ncRNAs), such as microRNAs, siRNAs and long non-coding RNAs (lncRNAs) accomplish critical roles in DENV infection [9-11]. In particular, lncRNAs have been recognized to be associated with various biological processes and involved in diverse types of human diseases [12]. Moreover, numbers of studies have investigated the expression of lncRNAs in dengue infection and identified several lncRNAs with perturbed expression [9,13]. Accumulating evidence strongly suggests that DENV and their hosts employ ncRNAs to modulate the outcome of DENV infection in their own favor [11]. For example, Wang et al. identified the differentially expressed lncRNAs in DENV1 and DENV2 serotypes infected L-02 cells and found that lncRNAs were implicated in transcription regulation and signal transduction [14]. LncRNA NEAT1 was observed with reduced expression in patients with dengue severe phenotype, which can potentially regulate the expression of interferon alpha inducible protein 27 (IFI27) [15]. However, we are still lack of knowledge about the comprehensive lncRNA expression profile in dengue infection, even more in the progression stages of dengue infections.

Identification of genes and lncRNAs involved in dengue infection would help in deciphering potential mechanisms responsible for the disease progression. Moreover, it is important to identify the potential regulatory factors that regulate the expression of genes and lncRNAs. N^6-methyladenosine (m6A) is the most abundant modification and it regulates RNA transcription, processing, splicing, degradation, and translation [16,17]. The m6A modification has been identified as an important regulator in regulating expression of lncRNAs [18,19]. However, limited studies have investigated the m6A modification during dengue infection [20,21]. It is important to understand how the host immune system reacts to infection and how the virus evades immune response for developing effective antivirals [22]. LncRNAs are emerging as critical regulators of gene expression in the immune system [23-25]. It is important to identify the lncRNA regulators correlated with immune response in dengue infection, which will provide insights into understanding lncRNA function and to advance identification of immunotherapy targets.

Dengue infection can also have varied manifestations. Liver involvement in dengue can cause painful hepatomegaly, transaminitis and jaundice [26]. Dengue viruses can infect human primary lung epithelia as well as lung carcinoma cells [27]. Dengue infection has been implicated with acute colitis and hematochezia [28], as well as acute demyelinating polyneuropathy [29]. However, a comprehensive picture among dengue infection and human complex diseases associations is still unknown.

To address these problems, we comprehensively analyzed the dynamic transcriptome during dengue disease progression and identified critical genes and lncRNAs with expression perturbations. We also analyzed the m6A regulation and identified the immune-related lncRNAs in dengue infection. Finally, we provided a comprehensive picture among dengue infection and 299 complex diseases based on network analysis.

2. Materials and methods

2.1. Transcriptome of dengue disease progression

Genome-wide transcriptome of dengue disease patients were obtained from Gene Expression Omnibus (GEO) under the accession number GSE94892 [30]. The datasets involved clinical samples from patients with fever for 1–3 days collected at the Calcutta School of Tropical Medicine (STM), Kolkata, and the University College of Medical Sciences (UCMS) and Guru Teg Bahadur (GTB) Hospital, Delhi from November 2014 and November 2016. Suspected DF cases were screened according to WHO diagnostic criteria (acute febrile illness on admission <7 days with one or more of headache, abdominal pain, retro-orbital pain, generalized aches and pains, fever, rash, petechiae, and signs of bleeding). Patients were classified according to the 2009 WHO classification scheme as the dengue infection without any warning sign (DI), dengue with a warning sign (DWS), severe dengue (DS) and other febrile illnesses (OFI). The mRNA profiling in the peripheral blood mononuclear cells (PBMCs) were profiled by RNA sequencing for all patients. We used OFI as control similar as one previous study [30]. In total, there were 39 samples, including 8 OFIs, 7 DIs, 9 DWSs and 15 DSs.

Raw RNA-Seq data were downloaded from Sequence Read Archive (SRA) and the genome annotation file was obtained from GENCODE (GRCh38) [31]. TopHat and Cufflinks were used for genome alignments, transcript assembly and abundance calculation [32]. The expression of protein-coding genes and lncRNAs were measured by Fragments Per Kilobase of exon model per Million mapped fragments (FPKM).

2.2. Identification of coding and non-coding RNAs with dynamic expression patterns

Based on the annotations in GENCODE, we obtained the expression profiles for protein-coding genes (PCGs) and lncRNAs. First, genes or lncRNAs with expression levels $= 0$ in $>70\%$ samples were excluded from our analysis. To identify the protein-coding genes

and lncRNAs with dynamic expression patterns during dengue diseases progression, we calculated the fold change between the later to the former stage. PCGs or lncRNAs with more than 1.2-fold were defined as up-regulated (U) and those with fold changes <1/1.2 were defined as down-regulated (D). Others were defined as maintained (M). In total, PCGs and lncRNAs were grouped into 27 clusters with different expression patterns. If the gene is upregulated during DI, upregulated during DWS, and maintained during DS, it is classified as up-up-maintained, i.e. UUM. The package Mfuzz in Bioconductor was used to visualize the expression of PCGs and lncRNAs [33].

2.3. RNA methylation of dengue infection

The modification N6-methyladenosine (m6A) affects rates of translation and degradation of mRNA transcripts. We analyzed m6A across the transcriptome following dengue virus infection using MeRIP-Seq. Three replicates for dengue infection were generated. Raw sequencing data were obtained from GEO under the accession number GSE130891 [21]. FASTQC was used for quality control and the adapters were removed by Trimmomatic [34]. STAR was used to align the reads to human reference genome (GRCh38) [35]. The peaks were identified by MACS2 [36]. We only used the peaks detected in at least two replicates and all peaks were mapped to PCGs or lncRNAs.

2.4. Protein-protein interactions between dengue and human

The protein-protein interactions between viral proteins and human proteins were collected from several public databases (including DenHunt [37] and DenvInt [38]) and literature [39,40]. In total, 8591 interactions among 10 viral proteins and 2874 human proteins were collected (Table S1).

In addition, we extracted the interactions among protein-coding genes and viral interactions from the curated PPIs. For lncRNAs, we calculated the Pearson correlation coefficients (PCCs) between lncRNAs and all PCGs. We selected the first 1 or 5 (top-1 or top-5) protein coding genes for each lncRNA as the candidate targets based on PCCs. The interactions were visualized by 'circlize' in R program [41].

2.5. Function enrichment analysis

To predict the function of PCGs and lncRNAs with dynamic expression patterns, we performed function enrichment analysis by Metascape [42]. For each lncRNA, we selected the first 1 or 5 (top-1 or top-5) PCGs with high correlation in expression. The lncRNA correlated top-1 or top-5 PCGs were subjected into Metascape for predicting the functions of lncRNAs. Moreover, the Gene Ontology (GO) terms were clustered and visualized by 'simplifyEnrichment' package in R program (https://bioconductor.org/packages/simplifyEnrichment/).

2.6. Immune cell infiltration of dengue patients

To estimate the proportion of immune cells infiltration in dengue patients, we used CIBERSORT for robust enumeration of cell subsets from tissue expression profiles [43]. The immune cell infiltration levels among different dengue infective stages were compared by Wilcox's rank sum test. Moreover, the Spearman Correlation Coefficient (SCC) between immune cell infiltrations and expressions of protein-coding gene or lncRNA were calculated.

2.7. Dengue infection and complex diseases association based on PPIs

To uncover the relationships between dengue infection and human complex diseases, we first collected the disease-related genes from one recent literature [44]. There were 3173 genes for 299 human complex diseases and these diseases were further clustered into 10 clusters [45]. We next evaluated the network-based overlap between the disease-related proteins and dengue infection-related genes. We first calculated the S_{vb} metric, where $S_{vb} < 0$ suggests a network-based overlap between the dengue infection-related gene encoded proteins v and proteins-associated with disease b. S_{vb} was calculated as:

$$S_{vb} = d_{vb} - \frac{d_{vv} + d_{bb}}{2}$$

where S_{vb} compares the average shortest distances between proteins within dengue infection-related genes encoded proteins d_{vv} or diseases-related proteins d_{bb}, to the average shortest distances d_{vb} between proteins encoded by dengue infection-related genes and disease proteins. Proteins associated with both conditions have $d_{vb} = 0$. Here, for protein-coding genes we used the genes with dynamic expression patterns, while for lncRNAs, we used the top correlated protein-coding genes in expression. The human protein-protein interactions were obtained from PCNet [46], which included 2,724,723 interactions among 19,779 proteins.

2.8. Availability of data and materials

All data generated or analyzed during this study are included in this published article. The gene expression profiles and clinical data can be found at GEO (https://www.ncbi.nlm.nih.gov/geo/). Software and resources used for the analyses are described in each

method section. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

3. Results

3.1. Dynamic transcriptome transition during dengue disease progression

To characterize dynamic transcriptional changes through the different stages of dengue infection, we first calculated the spearman correlation coefficients (SCC) among patients in different stages. We found that the transcriptome of patients in the same stage exhibited higher similarity than between stages (Figure S1). These observations suggested the dynamic alterations of transcriptome during dengue infection. We next classified the protein coding genes (PCGs) and lncRNAs with expression perturbations into different clusters (Figure S2). We first compared the expression of genes and lncRNAs between two adjacent stages. Genes exhibited higher expression in the later stage were defined as up-regulated and those exhibited lower expression were defined as down-regulated. We particularly focused the genes and lncRNAs that exhibited consistent expression patterns without opposite trend during infection. We identified 1349 PCGs exhibited consistently up-regulated (UUU) or down-regulated (DDD) transitions during dengue infection (Fig. 1A). There were 119 and 20 PCGs exhibited continuous up-regulation (UUU) or down-regulation (DDD) during three infective stages (see details in methods). Several genes have been demonstrated to be associated with infection (Table S2), such as CELSR3 [47], MAST1 [48], HSPG2 [49] and SCARF2 [50].

Moreover, we identified 786 lncRNAs that exhibited expression perturbations during dengue infection (Fig. 1B and Figure S3). In particular, 74 and 36 lncRNAs were up-regulation or down-regulation, including LINC00582, NKILA and RP11-87G24.3 (Table S3). Next, we visualized the expression of PCGs and lncRNAs across four stages (Fig. 1C and D). We found that the majority of PCGs and lncRNAs exhibited expression perturbation at the early stage of infection, such as from DI to DWS. These results suggest that the

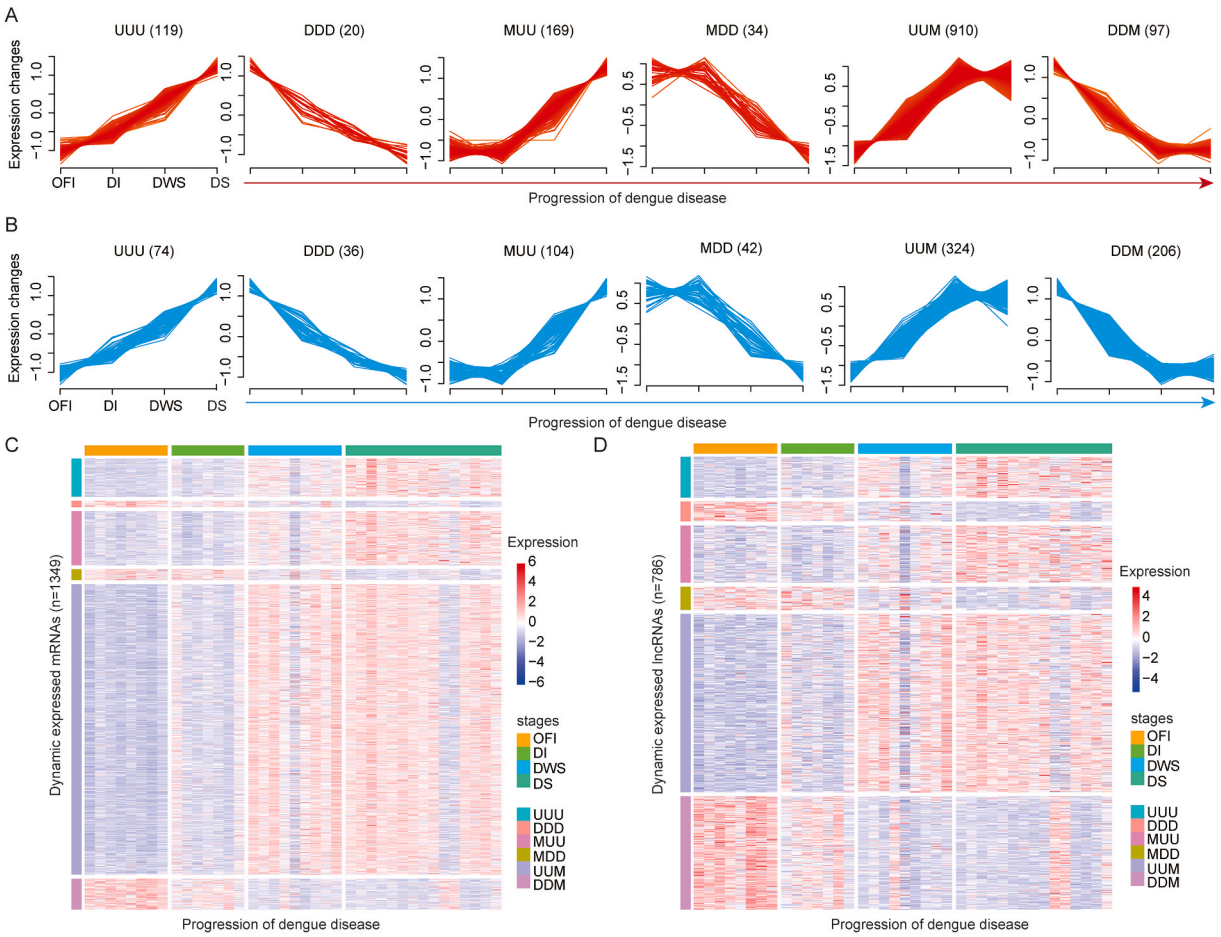

Fig. 1. Dynamic transcriptome alteration during dengue infection disease progression. A, Clustering of dynamically expressed protein-coding genes during disease progression. B, Clustering of dynamically expressed lncRNAs. C, Heat map showing the expression of dynamically expressed protein-coding genes. D, Heat map showing the expression of dynamically expressed lncRNAs.

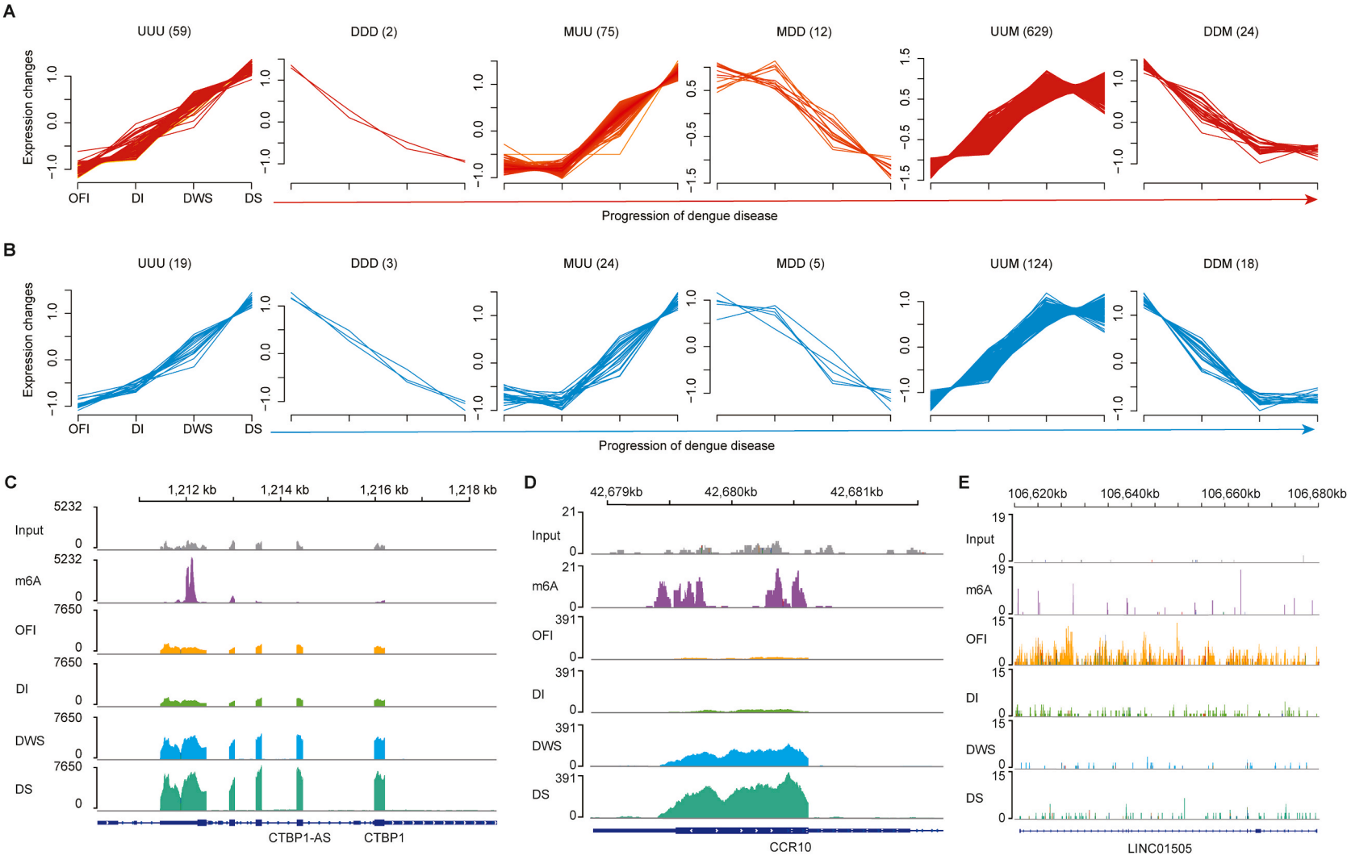

Fig. 2. LncRNA and mRNA expression regulated by m6A methylation. A, Clustering of dynamically expressed protein-coding genes, which were regulated by m6A methylation during disease progression. B, Clustering of dynamically expressed lncRNAs, which were regulated by m6A methylation during disease progression. C-E, Genome Browser view depicting m6A methylation and RNA expression distributions at the gene and lncRNA cluster. C for lncRNA CTBP-AS1, D for gene CCR10 and E for lncRNA LINC01505.

transcriptome of host changed greatly to response the viral infection at the infective stages.

3.2. Dynamically expressed PCGs and lncRNAs are regulated by RNA methylation

Gene expressions are regulated by various types of regulators, such as transcription factors, miRNAs and RNA methylation [51,52]. The roles of m6A methylation have been implicated in virus-host interactions [53-55]. Thus, we next investigated to what extent the PCGs and lncRNAs were regulated by RNA methylation during dengue infection. In total, we identified 9598 PCGs and 2047 lncRNAs were with RNA methylation peaks (Table S4 and Table S5). Particularly, there were 801 PCGs and 193 lncRNAs were with consistently expression perturbations (Fig. 2A and B).

We next analyzed the 83 PCGs and lncRNAs with UUU and DDD expression patterns in detail. Based on literature mining, we identified ten PCGs and lncRNAs that were correlated with diseases or immune regulation (Table 1). For instance, the lncRNA CTBP1-AS has been demonstrated to promote cell proliferation, invasion and migration, as well as inhibit cell apoptosis [56]. We found that CTBP1-AS exhibited continuously up-regulated expression during dengue infection (Fig. 2C), and there was an m6A methylation peak in CTBP1-AS. Moreover, the adjacent protein-coding gene CTBP1 also exhibited higher expression in DS stage (Fig. 2C). CCR10, a member of chemokine receptor has been found to play crucial roles in various diseases by stimulating the invasion and migration [57]. We found that CCR10 exhibited significantly high expression in DWS and DS, and there were numbers of RNA m6A methylation peaks located in the CCR10 regions (Fig. 2D). RNA m6A methylations have been demonstrated to play important roles in regulation of gene expression [55]. These observations suggested that m6A methylation might play important roles in regulation the expression of CCR10 in dengue infection.

Another example is the lncRNA RP11-308N19.1 (LINC01505), which exhibited continuously down-regulated expression during dengue infection (Fig. 2E). LINC01505 has been implicated in cancer by tumor genomes analysis [58]. Moreover, GNG7, PLXNA3, MAFG-AS1 and SOX7 were also found to be associated with cell proliferation and migration [59-61]. We also observed that there were several m6A peaks around GNG7, MIXL1 and SIX5 (Figure S4), suggesting that these genes were also regulated by RNA methylation. All these results suggest that dynamically expressed genes were regulated by RNA methylation, which plays important roles during dengue infection.

3.3. Dynamically expressed PCGs interact with viral proteins frequently

The protein-protein interactions (PPIs) among human and virus mediate viral infection and host immune response [62]. Therefore, we collected the comprehensive PPIs among dengue proteins and human proteins from literature and database. We found that the PCGs exhibited dynamic expression during dengue infection frequently interact with viral proteins (Fig. 3A). For example, ARHGAP33 with UUU expression pattern (Figure S5A) interacts with five dengue proteins (prM, C, NS2B, NS5 and NS4B). Moreover, the NS5 proteins of dengue interact with 37 human proteins encoded by genes with dynamic expression patterns (Table S6). The dengue virus

Table 1
Protein-coding genes and lncRNAs with dynamic expression and correlated with immune regulation.

Ensemble IDs	Gene symbols	Locus	Types	Descriptions	PMIDs
ENSG00000280927	CTBP1-AS	4:1,210,119–1218,591	UUU_lncRNA	promote cell proliferation, invasion and migration, as well as inhibit cell apoptosis	PMID: 31165609, PMID: 25552498
ENSG00000234323	RP11-308N19.1	9:106,616,058–106,679,802	DDD_lncRNA	implicated in cancer by tumor genomes analysis	PMID: 28128360
ENSG00000184451	CCR10	17:42,678,888–42,683,917	UUU_mRNA	a member of chemokine receptor plays crucial roles by stimulating the invasion and migration	PMID: 28830025
ENSG00000176533	GNG7	19:2,511,218–2702,709	UUU_mRNA	cell proliferation and migration	PMID: 33864871, PMID: 30945310, PMID: 9600093
ENSG00000130827	PLXNA3	X:154,458,280-154,477,779	UUU_mRNA	cell proliferation and migration	PMID: 21925246
ENSG00000265688	MAFG-DT	17:81,927,828–81,930,753	UUU_lncRNA	cell proliferation and migration	PMID: 32079456, PMID: 31002134, PMID: 33989902, PMID: 33336731
ENSG00000171056	SOX7	8:10,723,767–10730,512	UUU_mRNA	cell proliferation and migration, SOX7 regulates MAPK/ERK-BIM mediated apoptosis in cancer cells	PMID: 31332289, PMID: 23290134
ENSG00000185155	MIXL1	1:226,223,617–226,227,054	UUU_mRNA	Transcription factors that regulate cell fate during development	PMID: 18032708
ENSG00000177045	SIX5	19:45,764,784–45,769,226	UUU_mRNA	High expression of six1-5 was associated with a greater likelihood of tumorigenesis	PMID: 27821176
ENSG00000175832	ETV4	17:43,527,843–43,546,340	UUU_mRNA	ETV4 promotes the progression of gastric cancer through regulating KDM5D, ETV4 is a theranostic target in clear cell renal cell carcinoma	PMID: 32196595, PMID: 32305558

A
Fold changes
2
1.2
1/1.2
0
UUM
UUU
VP
DDM
MUU
DI/OFI
DWS/DI
DS/DWS
B
Fold changes
2
1.2
1/1.2
0
UUM
MUU
MDL
UUU
VP
DDM
DDD
mRNA
DI/OFI
DWS/DI
DS/DWS
C
Kruskal−Wallis, p = 9.6E−16
2.2E−13
7.6E−05
log2(degree)
15
10
5
0
lncRNA_top1
mRNA
others
D
Kruskal−Wallis, p = 2.2E−11
5.9E−11
0.00012
log2(betweenness)
0
−10
−20
−30
lncRNA_top1
mRNA
others
E
Kruskal−Wallis, p < 2.2E−16
9.6E−16
6.8E−08
log2(closeness)
−16.4
−16.6
−16.8
−17.0
lncRNA_top1
mRNA
others

Fig. 3. Dynamically expressed genes and lncRNAs interact with viral proteins. A, Circos plot showing the interactions among dynamically expressed genes and dengue viral proteins. The heat map showing the fold changes of expression during disease progression. B, Circos plot showing the correlations among dynamically expressed lncRNAs and correlated top 1 coding genes, as well as interactions with dengue viral proteins. The heat map showing the fold changes of expression during disease progression. C, Degree distribution of lncRNA-correlated genes, mRNAs and other genes in human interactome. D, Betweenness distribution of lncRNA-correlated genes, mRNAs and other genes in human interactome. E, Closeness distribution of lncRNA-correlated genes, mRNAs and other genes in human interactome.

NS5 protein has been identified as a target for drug discovery [63]. Investigation of the interactions among viral proteins and human proteins provided a method to understanding the roles of genes with expression perturbations.

Moreover, we investigated the interactions among lncRNAs and viral proteins. As lncRNAs usually regulate the expression of PCGs, we calculated the correlations between expressions of PCGs and lncRNA. PCGs were ranked based on the correlation, and we next

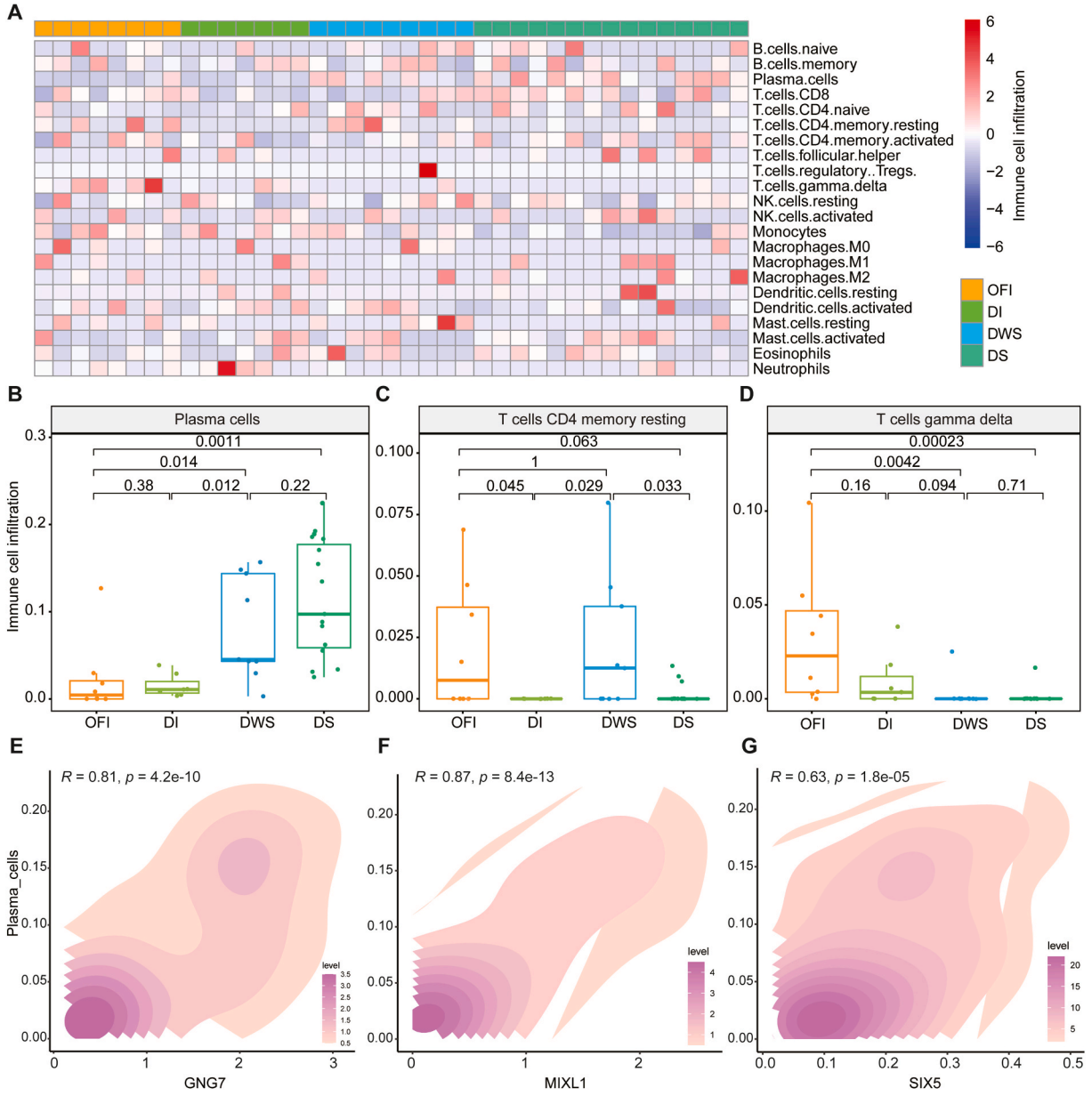

Fig. 4. Immune microenvironment alterations during dengue infection progression. A, Heat map showing the immune cell infiltration in patients with dengue infection. B-D, Boxplots showing the distribution of immune cell infiltrations. B for plasma cells, C for CD4 memory resting T cells and D for gama-delta T cells. P-values for Wilcoxon's rank sum tests. E-G, Representative examples showing correlations between gene or expression and immune cell infiltration levels.

identified the top-1 (or first one) PCG that was mostly correlated with lncRNAs in expression. We found that the lncRNA correlated genes also frequently interact with dengue proteins (Fig. 3B). For example, we found that PARP1, a gene encoding DNA damage-related proteins, was correlated with the expression of CTD-2260A17.2 (Figure S5B-5C, R = 0.86 and p = 1.3E-12), which exhibited higher expression in DI and DWS. Interaction analysis revealed that PARP1 can interact with 9 dengue proteins (Table S6). Previous study had been demonstrated that PARP1 can promote virus replication by degradation of host type I IFN receptor [64]. Another example is SLC1A5, which was correlated with expression of CTB-147N14.6 (Figure S5D-5E, R = 0.97 and p = 2.2E-16). We found that SLC1A5 can interact with 8 dengue proteins (Table S6). Evidence has suggested that virus can activate and require the Myc network to up-regulate SLC1A5, leading to increased glutamine uptake for viral infections [65]. We obtained the similar results for lncRNAs based on top-5 most correlated genes (Figure S6 and Table S6). We identified some genes associated with DNA damage, such as PARP1, STRA13, RAD51D, RAD23A and TELO2 [66,67]. These findings provide novel insight into interactions between dengue virus and the host immune response.

The function of proteins can be represented by their locations in human PPI network [68-70]. Thus, we next investigated the

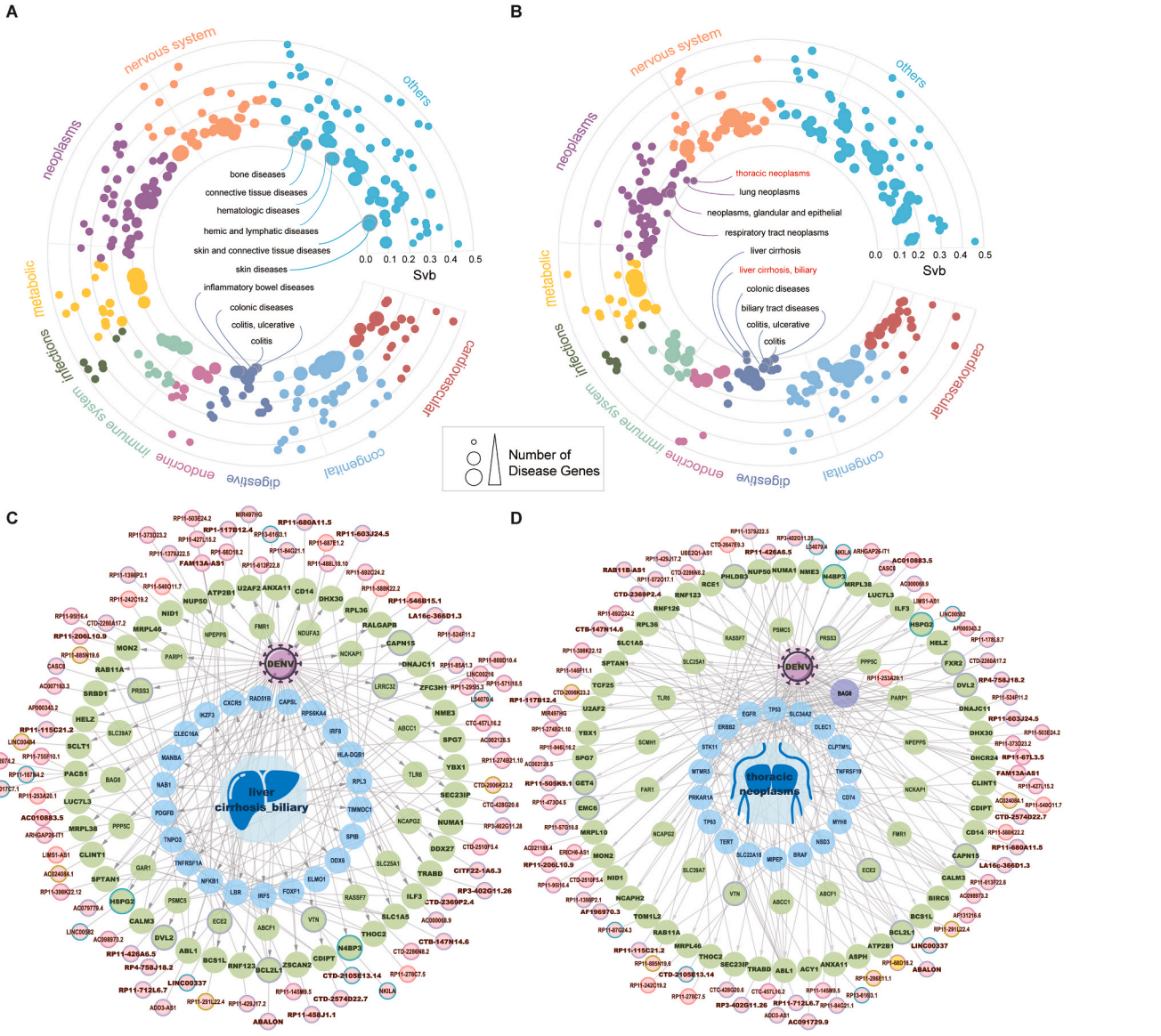

Fig. 5. DENV infection associated with human complex diseases. A, Disease comorbidity measured by the network overlap between dengue infections-related protein coding genes and 299 diseases. The dots represent diseases whose radius reflects the number of associated diseases genes. The diseases closest to the center, whose names are marked are expected to have higher comorbidity with viral infection. B for diseases associations based on lncRNA-correlated genes. C-D, Network visualization showing the protein-protein interactions among DENV viral interacting proteins and diseases associated proteins. C for DENV infection and liver cirrhosis biliary and D for DENV infection and thoracic neoplasms.

location of dengue interacting proteins that were with expression perturbations during infection. We divided all the proteins in PPI network into three groups: dengue targeting proteins encoded by coding genes with expression perturbation, proteins encoded by lncRNAs correlated genes and other proteins. We found that dengue targeting proteins have significantly higher degrees, betweenness and closeness in PPI network than other proteins (Fig. 3C–E, all p-values <0.01). We obtained the similar results for lncRNAs based on top-5 most correlated genes (Figure S7). These results suggest that dengue viruses are likely to target proteins that are located in the central of network or those play important roles in information spread for replication.

3.4. Dynamically expressed PCGs and lncRNAs are involved in immune regulation

To identify the functional pathways perturbed during dengue infection, we next characterized the function of PCGs or lncRNAs with dynamic expression patterns. We first performed functional enrichment analysis for genes or lncRNA-correlated genes. We found that the PCGs were significantly enriched in proliferation regulation, signaling pathways and immune response (Figure S8A). Cell cycle process was significantly enriched by protein-coding genes with dynamic expression patterns (Table S7), which is important for viral infection [71].

Moreover, we predicted the functions of lncRNAs based on their correlated coding genes. We found that lncRNAs play important roles in cell cycle regulation, organization regulation, and immune cell regulation (Figure S8B and Table S7). Similar results were observed when using the top-5 most correlated genes (Figure S9 and Table S7). Accumulating evidence strongly suggested that dengue viruses and their hosts employ lncRNAs to modulate the outcome of infection in their own favor [11]. These results suggested that dengue viruses selectively suppress immune responses and cell cycle pathways in the cells it infects, that may have important consequences for virus infection and transmission.

3.5. Association of PCGs and lncRNAs with immune cell infiltrations

Infiltrating of immune cells is closely associated with clinical outcome. However, we are still lack of knowledge whether the immune cell infiltrations were altered during dengue infection. Thus, we estimated the immune cell infiltration levels in each patient based on CIBERSORT. We found that the immune cell infiltrations were highly variable among patients (Fig. 4A). In particular, we found that plasma cells infiltrations increased during dengue infection (Fig. 4B, Wilcoxon's rank sum tests) and CD4 memory resting T cells exhibited higher infiltration levels in DWS (Fig. 4C, Wilcoxon's rank sum tests). Plasma leakage is an important biomarker for early detection of dengue hemorrhagic fever [72,73], which is consistent with our observations. Moreover, we observed decreased γδ T cells infiltrations during dengue infection (Fig. 4D, Wilcoxon's rank sum tests), which have been demonstrated to play important roles in viral infection [74].

Next, we investigated the correlation between gene expression and immune cell infiltrations in dengue infection. We found that the expressions of several genes were significantly correlated with plasma cells infiltrations, such as GNG7 (Fig. 4E, $R = 0.81$ and $p = 4.2E\text{-}10$), MIXL1 (Fig. 4F, $R = 0.87$ and $p = 8.4E\text{-}13$) and SIX5 (Fig. 4G, $R = 0.63$ and $p = 1.8E\text{-}5$). Emerging evidence has suggested that GNG7 plays a non-ignorable role in the progression of COVID-19 [75]. SIX5 has been found to be required to activate immunity [76]. These results suggested that dynamically expressed PCGs and lncRNAs are associated with immune cell infiltrations and play important roles in dengue infection.

3.6. Complex diseases associated with dengue infection

Emerging evidence has revealed that complications and comorbidities of dengue infection [77,78]. We next systematically evaluated the human complex disease associations with dengue infection based on the state-of-art network proximity measure. Based on the PCGs, we found that dengue infection was more likely correlated with digestive diseases and skin diseases (Fig. 5A). Moreover, several neoplasms and digestive diseases were correlated with dengue infection based on lncRNA-correlated coding genes (Fig. 5B). In particular, liver cirrhosis and thoracic neoplasms were with smaller Svb network measures. Moreover, we identified 14 human complex diseases were all associated with dengue infection in coding gene-based, lncRNA top-1 and top-5 based analyses (Figure S10), such as gastroenteritis, colitis, and neuromuscular diseases.

Network visualization can allow us further to show the protein-protein connections between dengue infection and other human complex diseases. We thus visualized the PPIs among dengue infection associated genes and liver cirrhosis associated genes (Fig. 5C). Dengue has been suspected as a cause of liver failure in endemic areas, where no etiological cause is discernible [26]. Our analysis revealed that dengue infection-associated proteins frequently interact with liver cirrhosis-associated proteins, which may provide a network-view for explaining the etiological cause. Moreover, thoracic neoplasms were observed to be associated with dengue infection via PPIs (Fig. 5D). Dengue viruses have been found to infect human primary lung epithelia as well as lung carcinoma cells, and can also induce the secretion of IL-6 and RANTES [27]. Moreover, we also revealed the close association between dengue infection-associated gastroenteritis diseases and neuromuscular diseases (Figure S11). All these results suggest that identifying the dynamically expressed genes and lncRNAs helps understanding the association between dengue infections with other human complex diseases.

4. Discussion

In this study, we comprehensively characterized the dynamic transcriptome during dengue infection disease progression. We identified numbers of PCGs and lncRNAs with expression perturbations during dengue infection progression. In particular, we found

that these genes also exhibited altered expression in dengue when compared with healthy donors (Figure S12). Moreover, the expression of PCGs and lncRNAs were potentially regulated by m6A methylation. Further functional enrichment analysis revealed that these PCGs and lncRNAs were involved in regulation of signaling pathway and immune response. Moreover, they were located in the central regions of human interactome. These observations suggested their important roles in dengue infection. Several PCGs and lncRNAs were correlated with immune cell infiltrations. Finally, we charted the landscape of dengue infection with 299 human complex diseases, providing insights into the dengue comorbidities.

It would be nice to compare the levels of proteins/RNAs from the same patient during all stages of disease progression. However, it is very difficult to obtain such datasets currently. It is usually to analyze the patients in different stages. Moreover, it would be nice to see the expression levels of proteins from both viral and host differed during the treatment. With the development of sequencing technologies and large number of datasets for dengue infection, our analysis provided a computational framework for such analysis, and can be applied to such datasets in the future.

A major unmet need in dengue infection management is the development of effective antiviral drugs. An important approach to using antivirals to treat dengue is to modulate the immune system of the host. In this study, we systematically characterized the immune microenvironment during dengue infection progression and identified numbers of lncRNAs or genes associated with immune cell infiltration. For example, the expressions of GNG7, MIXL1 and SIX5 were significantly associated with plasma cells infiltration. Therefore, these biomarkers provided good candidates for further experimental validation.

Although great efforts have been made in the transcriptome research of dengue infection, many questions remain to be clarified in the future. In particular, host immune response plays important roles in dengue infection; however, the dynamic immune ecosystem during dengue infection disease progression is largely unknown. Bulk transcriptome and epi-transcriptome analyses in dengue infection were performed and numbers of biomarkers were identified [21,79,80]. Lack of the comprehensive knowledge has impeded the precise diagnostic test of dengue infection. With the emerging single cell sequencing data [81], we will reveal the dynamic immune ecosystems that are highly associated with the progression of dengue infection. Moreover, we can also identify the lncRNAs or genes biomarkers based on single cell data, which will provide novel insight into the underlying mechanism of dengue infection.

In summary, our study reveals dynamic transcriptome alterations that are highly associated with the progression of dengue infection. The PCGs and lncRNAs and their regulated pathways, as well as the disease associations identified here should be considered for further validation in larger prospective cohorts. The resulting data presented here serve as a valuable resource for understanding lncRNA function and to advance identification of immunotherapy targets for dengue infection.

Ethics approval and consent to participate

Not applicable.

Authors' contributions

J.Y., Y.L. and X.L. designed this study. Y.Z., J.G. and Y.G. analyzed the data and interpreted the results. T.P., G.X. and S. L. performed the pathways and network analyses. Y.L., Y.Z. and J.G. wrote and edited the manuscript, and all authors read and approved the manuscript with contributions from other authors.

Additional information

All data generated or analyzed during this study are included in this published article. The gene expression profiles and clinical data can be found at GEO (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE94892. Software and resources used for the analyses are described in each method section.

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Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2022.e12690.

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