Dual RNA-Seq of H5N1 Avian Influenza Virus and Host Cell Transcriptomes Reveals Novel Insights Into Host-Pathogen Cross Talk

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H5N1 avian influenza virus (AIV) is a highly pathogenic influenza virus that poses a substantial threat to poultry production and public health. A comprehensive understanding of host–pathogen interactions for AIV requires knowledge of gene expression changes in both the pathogen and the host upon infection. We report the use of dual RNA sequencing technology to uncover trends in gene expression in H5N1 AIV and chickens (DF1 cells) during the course of infection. The expression of all viral genes increased continuously from 0 to 20 h post infection. We also identified 2,762 differentially expressed host genes during infection. Pathway analysis found that genes related to the signaling pathways of DNA replication, T cell activation, NF-kappa B signaling pathway, and RNA degradation were significantly enriched. We demonstrated that the cis-acting lncRNA MSTRG.14019.1 targeted CSE1L and may affect virus replication. This study provides a more comprehensive and detailed understanding of host-virus interactions at the RNA level during the course of H5N1 AIV infection.

Keywords: dual RNA sequencing, H5N1, chicken, CSE1L, lncRNA

INTRODUCTION

Influenza A virus (IAV) is a member of the Orthomyxoviridae family and is a significant threat to public health. Moreover, highly pathogenic H5N1 avian strains also threaten to cause worldwide pandemics and substantial economic losses (Schrauwen et al., 2014). IAV is a segmented, single-stranded, negative-sense RNA virus, with a genome consisting of eight gene segments that encode at least 11 proteins (Wise et al., 2009). Upon infection by influenza, host cells detect viral RNA through pathogen sensors, and the major gene products of the influenza virus mediate the viral life cycle and modulate cellular processes (Dou et al., 2018).

A comprehensive understanding of host-pathogen interactions requires knowledge of the associated gene expression changes in the pathogen and the host. Dual RNA sequencing (dual...
RNA-seq technology allows for the parallel analysis of host and pathogen transcriptomes, which facilitates the detection of gene expression changes in the pathogen and host simultaneously (Westermann et al., 2012). Most importantly, the dual RNA-seq approach allows genes from both host and pathogen to be monitored at different time points throughout infection, from initial contact through to invasion, and finally, the manipulation of the host (Westermann et al., 2016).

Pathogen infection triggers a series of dynamic reactions in the host, and eventually leads to changes in the gene expression patterns of the pathogen and the host. Such changes may lead to the adaptation and tolerance of the pathogen, or may trigger the host immune response to eliminate the pathogen. A comprehensive understanding of host and pathogen transcriptome information will help us to identify virulence factors of new pathogens, pathogen-associated molecular patterns, or new host pathways that target specific pathogens, thus, furthering the understanding of interactions between pathogens and hosts (Baddal et al., 2015; Ranaware et al., 2016).

Avian derived cell lines such as chicken embryonic fibroblasts (DF1) can be used to propagate influenza viruses because they express α-2,3-linked sialic acid receptors, which are preferentially targeted by avian-adapted viruses (Lee et al., 2008). Moreover, immortalized cell lines provide a suitable platform for generating stable cell lines that can be used for virus propagation (Chungu et al., 2021). Here, the dynamic regulation of virus and host gene expression in H5N1 AIV-infected chicken DF1 cells was studied using dual RNA-seq. After H5N1 AIV infection of chicken DF1 cells for 0, 6, 12, and 20 h, the expression of different viral genes was increased. A total of 2,762 differentially expressed host genes were also identified at different time points. We further explained the role of the differentially expressed genes (DEGs) in viral infection using pathway enrichment analyses, and compared those findings to published proteomics analyses, and compared those findings to published proteomics data to screen key genes associated with the influenza promoting that affect viral replication. Notably, the cis-acting lncRNA MSTRG.14019.1 targeted and regulated the influenza promoting that affect viral replication. Thus, these results suggest that lncRNA MSTRG.14019.1 may inhibit the replication of H5N1 AIV by inhibiting the expression of CSEIL.

**MATERIALS AND METHODS**

**Cells and Virus**

DF1 cells and Madin–Darby canine kidney (MDCK) cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 µg/ml streptomycin and 100 U/ml penicillin at 37°C, under a humidified atmosphere of 5% CO₂. The highly pathogenic H5N1 strain A/mallard/Huadong/S/2005 (SY) (Tang et al., 2009) was propagated in 10-day-old specific-pathogen-free embryonic chicken eggs. All experiments involving live viruses were performed in a biosafety cabinet with HEPA filters in a biosafety level 3 laboratory at Yangzhou University, Yangzhou, China.

**Viral Infection and RNA Sample Collection**

DF1 cells were incubated in a 6-well plate at 37°C and 5% CO₂. The cells were cultured until they achieved 90% confluence. Four 6-well plates containing DF1 cells were cultured under the same conditions. The uninfected cells were collected directly and stored in liquid nitrogen. The three remaining plates were infected with 0.1 PFU at 37°C and incubated for 1 h at room temperature. The inoculum was then removed and replaced with DMEM lacking FBS. Cells were collected at 6, 12, and 20 h after infection, and stored in liquid nitrogen. Total RNA was extracted using an RNeasy minikit (Qiagen, Valencia, CA, United States), followed by DNase treatment.

**Strand-Specific Total RNA Library Preparation and Sequencing**

All samples had an RNA Integrity Number (RIN) of > 8 (Agilent Technologies, Santa Clara, CA, United States). Starting with 1 µg of DNA-free total RNA extracted from mock- and H5N1-infected DF1 cells, rRNA was first removed through two rounds of selection using a Ribo-Zero RNA removal kit (Epigeme, Madison WI, United States). The resulting RNA was then used to construct an RNA-seq library using the Illumina Truseq strand-specific library preparation kit (Illumina, Inc., San Diego, CA, United States). To obtain the final sequencing library, 15 cycles of Polymerase Chain Reaction (PCR) were performed using Phusion Hot Start high-fidelity DNA polymerase (Finnzymes, Espoo, Finland). RNA-seq libraries were size selected for the retention of insert fragments between 100 and 300 base pairs (bp). Mature small RNAs (including sRNAs) were not considered in our analysis. For each sample replicate, we obtained ~30 million paired 50-mer reads (with three biological replicates per sample) using the Illumina Hiseq-2000 platform (Illumina, Inc.).

**Read Mapping and Differential Expression Analysis of RNA-Seq Data**

For the chicken host transcriptome analysis, reads were aligned in paired-end mode to a chicken genome (Ensemble gga6.0) using the HISAT2 1 using the default settings. Reads that were not mapped to Ensemble gga6.0 were mapped to influenza genomes: A/goose/Guangdong/1/1996(H5N1), 2 Viral reads were mapped to viral genomes using Bowtie2 3 (Fabozzi et al., 2018). Differential expression was evaluated for each time point using edgeR (Lun et al., 2016). Differential expression was detected using the following thresholds: FDR < 0.05 & |log2FC| ≥ 1.

1https://ccb.jhu.edu/software/hasit2/index.shtml
2https://www.ncbi.nlm.nih.gov/genome/genomes/10290?
3http://bowtiebio.sourceforge.net/bowtie2/index.shtml
Identification of Candidate IncRNAs and Target Gene Prediction

The low-quality reads, adaptor sequences, empty reads, and ribosomal (r)RNA reads were removed from the raw data to obtain high-quality IncRNAs. The read coverage of transcripts was calculated using Stringtie (1.3.3) (Pertea et al., 2015), and those with reads shorter than 200 nt were eliminated. The Coding-Non-Coding-Index (CNCI) (Sun et al., 2013) (score < 0), Coding Potential Calculator (CPC) (Kong et al., 2007) (score < 0), and Pfam-scan (Finn et al., 2016) (E-value < 0.001) were used to assess the coding potential of the remaining transcripts. Transcripts identified with coding potential by any of the three tools were filtered out.

The identified IncRNAs were classified by FEELnc (Wucher et al., 2017). According to the location with the corresponding gene, IncRNAs were classified as intergenic or intragenic. The intragenic IncRNAs were further subclassified into four categories: (1) sense intronic IncRNAs, (2) antisense intronic IncRNAs, (3) sense exonic IncRNAs, and (4) antisense exonic IncRNAs.

Differentially expressed IncRNAs (DE-IncRNAs) were selected for target prediction. The cis role of IncRNAs was their action on neighboring target genes (Ørom et al., 2010). The DEGs located within a 100 kb distance of the DE-IncRNAs were selected as potential target genes. The Pearson correlation test was used to calculate the correlation coefficients between IncRNAs and their potential target genes. RNAplex (Tafer and Hofacker, 2008) was used to predict the target genes of tran-acting IncRNAs. RNAplex can quickly identify the possible hybridization sites with the query RNA sequence (IncRNA) in the RNA database (i.e., the target genes of trans-acting IncRNAs).

Gene Ontology Term Enrichment and Pathway Enrichment Analyses

Gene ontology enrichment and pathway enrichment were conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang et al., 2009).

Quantitative Real-Time Polymerase Chain Reaction

The efficiency of the knockdown of CSEIL expression was confirmed by quantitative real-time PCR. Total RNA was isolated from DF1 cells using TRIzol reagent (Tiangen). One microgram of total RNA per sample was reverse transcribed into cDNA using the FastQuant RT Kit (Tiangen). We next used the ABI Prism 7500 system (Applied Biosystems) with the One Step SYBR PrimeScript RT-PCR Kit II (Takara) to analyze the expression of CSEIL. The expression of each gene, relative to that of glyceraldehyde-3-phosphate dehydrogenase, was calculated using the $2^{-\Delta\Delta Ct}$ method.

RNA Interference

All of the siRNAs used in this study were designed and synthesized by Guangzhou Ruibo (Guangzhou, China). At a confluence of 90% in 6-well plates, DF1 cells were transfected with 100 nM of siRNA targeting the chicken CSEIL gene (Gene ID: 395958); siCSEIL, sense 5'-GCAAGAATCCATCTGTTA-3'). The negative control siRNA was a scrambled siRNA for CSEIL (siNC, sense 5'-GUGAAGAAUCGUAAUUTT-3'). All siRNAs were transfected into DF1 cells using Lipofectamine 3000 (Life Technologies).

Infectious Titer of Influenza Virus (TCID50 Assay)

The TCID50 assay was used to evaluate progeny virus production. After transfection with siRNA targeting the CSEIL gene or the negative control siRNA, all of the DF1 cells were infected with the SY virus at a multiplicity of infection of 0.1 PFU. At 1 h post infection (1 hpi), the medium was replaced with DMEM lacking fetal bovine serum. Conditioned medium was then collected at 12 and 24 hpi to measure viral titers. Viral titers were determined using the agglutination assay after growth in MDCK cells. The MDCK cells were seeded in 96-well plates and infected after reaching 85% confluence. Cells were washed twice with phosphate-buffered saline (PBS) and infected with a series of virus dilutions, and incubated for 72 h, as described above. Agglutination assays were performed in round-bottomed 96-well plates using 1% chicken red blood cells in PBS.

Statistical Analysis

The viral titers were calculated as the means ± standard deviations from three independent experiments. Independent-samples t-tests were used to analyze the TCID50. For all tests, a $p \leq 0.05$ was considered statistically significant.

RESULTS

Overview of the Dual RNA-Seq Data From H5N1-Infected Chicken DF1 Cells

We infected chicken DF1 cells with the highly pathogenic H5N1 strain, A/mallard/Huadong/S/2005 (SY). Total RNA was extracted from virus-infected cells at 0, 6, 12, and 20 hpi, and subjected to a dual strand-specific RNA-seq strategy using the Illumina HiSeq instrument. Figure 1A shows the distribution of host and viral read counts for each sample. After viral infection, the viral read counts increased gradually from 0.02 to 62.44%. Conversely, the number of read counts decreased gradually from 95.71 to 10.81%. Following viral infection, the unmatched read counts increased continuously (Figure 1A). The expression of negative-strand RNAs (vRNAs) versus positive-strand RNAs (cRNA/mRNA) from each gene segment was evaluated by normalizing the read coverage of viral gene segments to the total number of chicken reads (Figure 1B). To understand the different characteristics between different time points after infection, we conducted cluster analyses and principal component analyses (PCA) of the differentially expressed influenza virus RNA, and chicken mRNA and IncRNA. Hierarchical clustering revealed that three virus infection groups and the control group were well distinguished in virus RNA and chicken mRNA and IncRNA, with all subjects correctly
RNA-Seq Identified Differentially Expressed mRNAs

A total of 2,762 genes were differentially regulated following H5N1 AIV infection in chicken DF1 cells. We then analyzed the number of DEGs, including up-regulated genes and down-regulated genes, between each pair of time points following infection (Table 1). Heat maps and line charts were generated to aid in the visualization of gene expression patterns (Figures 2A,B). The results also showed that the DEGs mainly conformed to four expression patterns: (1) they were first decreased and then increased, (2) first increased and then decreased, (3) continuously increased, and (4) continuously decreased (Figures 2A,B). Comparative analyses between six groups of DEGs (0 hpi vs. 6 hpi, 0 hpi vs. 12 hpi, 0 hpi vs. 20 hpi, 6 hpi vs. 12 hpi, 6 hpi vs. 20 hpi and 12 hpi vs. 20 hpi) were performed (Figure 2C). There were several DEGs shared between different groups, however, many more genes were expressed at different time points following infection (Figure 2C and Supplementary Table 1). Next, we performed functional classification analyses of the DEGs using DAVID Bioinformatics Resources. Gene Ontology (GO) enrichment of the DEGs included positive regulation of intrinsic apoptotic signaling pathway, DNA replication, nuclear envelope, and ATP-dependent helicase activity (Table 2). The DEGs related to pathways for DNA replication, T cell activation, NF-kappa B signaling, and RNA degradation were also significantly enriched (Figure 2D). We also analyzed the
### TABLE 1 | Differentially expressed genes at different time points post infection in chicken DF1 cells.

| Group       | All  | Up   | Down |
|-------------|------|------|------|
| 0 h vs. 6 h | 67   | 29   | 38   |
| 0 h vs. 12 h| 1,144| 835  | 309  |
| 0 h vs. 20 h| 2,028| 900  | 1,128|
| 6 h vs. 12 h| 1,039| 774  | 265  |
| 6 h vs. 20 h| 2,037| 881  | 1,156|
| 12 h vs. 20 h| 821 | 274  | 547  |

pathway enrichment of DEGs at different infection time points (Supplementary Table 2).

### RNA-Seq Identified Differentially Expressed lncRNAs

A total of 1,416 lncRNAs were found to be differentially expressed between the uninfected group and the infected group, or between groups at different time points post infection (Table 3). Similar to the mRNA results, heat maps were generated to aid the visualization of the lncRNA expression patterns (Figure 3). The results showed that the DE-lncRNAs exhibited three main expression patterns: (1) they were first increased and then decreased, (2) continuously increased, and (3) continuously decreased (Figure 3).

### Target Genes of lncRNAs

A total of 6,586 and 4,788 pairs of regulatory relationships were identified by the cis- and trans- predictive analyses, respectively (Supplementary Tables 3, 4). There were 1,128 pairs of targeted regulatory relationships between the DE-lncRNAs and their cis- and trans-target DEGs (Supplementary Table 5). The interaction networks of the DE-lncRNAs and their cis- and trans-target DEGs were also constructed (Supplementary Table 5 and Figure 4). The mapping of lncRNA-mRNA relationships showed that lncRNAs target mRNAs in a variety of ways. For example, 16 lncRNAs, including lncRNA MSTRG.10545.15 and lncRNA MSTRG.10545.20 simultaneously target SNAP47 mRNA. Additionally, lncRNA ENGALT00000081479 binds to six genes, including P4HB and ARHGDI, and many other lncRNAs target specific genes (Figure 4). GO enrichment

![Figure 2](image-url)
### TABLE 2 | Gene ontology enrichment of differentially expressed genes.

| Gene ontology                                  | Gene number | P-value |
|-----------------------------------------------|-------------|---------|
| **Biological process**                        |             |         |
| Positive regulation of intrinsic apoptotic signaling pathway | 7           | 0.0033  |
| Regulation of DNA-dependent DNA replication initiation | 4           | 0.01    |
| DNA replication                               | 15          | 0.011   |
| Negative regulation of insulin receptor signaling pathway | 8           | 0.016   |
| Maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) | 8           | 0.016   |
| **Molecular function**                        |             |         |
| Mitochondrion                                 | 122         | 0.000039|
| Extracellular exosomes                        | 254         | 0.000075|
| Myelin sheath                                 | 31          | 0.00043 |
| Centrosome                                    | 52          | 0.0011  |
| Cytosol                                       | 129         | 0.0025  |
| Membrane                                      | 114         | 0.0042  |
| Acrosomal vesicle                             | 9           | 0.0058  |
| Sperm flagellum                               | 6           | 0.0076  |
| Ubiquitin ligase complex                      | 15          | 0.0078  |
| Pwp2p-containing subcomplex of 90S preribosome | 4           | 0.01    |
| Endoplasmic reticulum lumen                   | 11          | 0.013   |
| Midbody                                       | 16          | 0.021   |
| Nuclear envelope                              | 15          | 0.033   |
| Focal adhesion                                | 47          | 0.037   |
| Golgi apparatus                               | 62          | 0.04    |
| Intracellular ribonucleoprotein complex        | 10          | 0.042   |
| Kinetochore                                   | 11          | 0.048   |

#### DISCUSSION

For many years, studies of cellular host responses to viral infections have focused on global changes at the mRNA level. More recently, dual RNA-seq has been used to simultaneously analyze the changes in host and pathogen mRNA expression profiles throughout the course of infection (Baddal et al., 2015; Fabozzi et al., 2018; Mika-Gospodorz et al., 2020; Pisu et al., 2020; Zhang et al., 2020; Karamitros et al., 2021; Penaranda et al., 2021). For example, Pisu et al. (2020) explored the in vivo molecular dynamics of Mtb infection of the cis- and trans-target genes of DE-lncRNAs included peptide antigen binding, regulation of T cell mediated immunity, the MHC class I protein complex, and positive regulation of T cell-mediated immunity (Supplementary Table 6). All mRNAs targeted by the differentially expressed lncRNAs were analyzed for pathway enrichment. The results revealed that 25 pathways were significantly enriched, while 16 pathways, including antigen processing and presentation, natural killer cell-mediated cytotoxicity, phagosome, and the Fc epsilon RI signaling pathway were implicated in the immune system or diseases (Table 4).

#### LncRNA MSTRG.14019.1 Targeting CSE1L Inhibits the Replication of H5N1 AIV

The analysis of DE-lncRNAs and their target genes revealed that lncRNA MSTRG.14019.1 targeted CSE1L. LncRNA MSTRG.14019.1 is located on the positive chain of chromosome 20 and is located upstream of CSE1L. The distance between CSE1L and lncRNA MSTRG.14019.1 was 75,117 bp, thus, indicating cis regulation. Compared to the uninfected group, the expression level of lncRNA MSTRG.14019.1 was significantly increased at 12 hpi, but it was significantly decreased at 20 hpi (Figure 5A). Contrary to the expression trend of lncRNA MSTRG.14019.1, the expression of CSE1L decreased significantly at 12 hpi (Figure 5B).

To study the role of CSE1L in the virus life cycle, we analyzed the effect of CSE1L downregulation on H5N1 AIV infection using small interfering RNA (siRNA)-mediated silencing. Real-time PCR confirmed that CSE1L expression was dramatically reduced in siRNA-treated DF1 cells, but not in cells treated with the scrambled control siRNA (Figure 5C). Subsequently, chicken DF1 cells treated with siRNA targeting CSE1L or with scrambled siRNA were then infected with H5N1 AIV. Culture supernatants were collected at 12 and 24 hpi, and titrated onto MDCK cells. As shown in Figure 5D, knockdown of CSE1L decreased the virus titer relative to that observed in scrambled siRNA-treated DF1 cells.

#### TABLE 3 | Differentially expressed lncRNAs.

| Group          | All | Up | Down |
|----------------|-----|----|------|
| 0 h vs. 6 h    | 29  | 17 | 12   |
| 0 h vs. 12 h   | 599 | 483| 116  |
| 0 h vs. 20 h   | 960 | 459| 501  |
| 6 h vs. 12 h   | 520 | 402| 118  |
| 6 h vs. 20 h   | 922 | 436| 486  |
| 12 h vs. 20 h  | 320 | 148| 172  |
by performing dual RNA-seq on Mycobacterium tuberculosis-infected, ontogenetically distinct macrophage lineages isolated directly from murine lungs. Similarly, the transcriptional response of intracellular *Pseudomonas aeruginosa* associated with urinary tract infections and their corresponding host cells were defined using simultaneous dual RNA-seq transcriptional profiling (Penaranda et al., 2021). Here, we used dual RNA-seq to characterize novel mechanisms of the interactions between the chicken DF1 cell line and H5N1 AIV, including those at the mRNA and lncRNA levels.

Through the transcriptome study of the interaction between H5N1 AIV and chicken DF1 cells, the dynamic change trend of virus and host genes at different infection time points has been more comprehensively detected, and lncRNA and mRNA with a targeted regulation relationship have also been found, which is helpful to analyze the virus host interaction and the new mechanism of host affecting avian influenza virus (AIV). In this study, H5N1 AIV infection resulted in 2762 DEGs in chicken DF1 cells. Pathway enrichment analyses of the DEGs identified significantly enriched pathways related to DNA replication, T cell activation, NF-kappa B signaling, and RNA degradation (Figure 2D). We further analyzed the pathway enrichment of DEGs at different time points post infection. The results showed that the inflammation mediated by chemokines and cytokine signaling pathways was significantly enriched in the DEGs at all infection time points, especially in the early stages of infection. The results revealed that during viral infection, cytokines and chemokines respond quickly to viral stimuli, thus, indicating that cytokines and chemokines, and their related signaling pathways play an important role in the process of H5N1 AIV infection,
and may be the main antiviral factors during the early stages of viral infection. It has been reported that H5N1 AIV can significantly increase the levels of cytokines and chemokines in host cells compared with that of H1N1 and H7N2 influenza viruses (Huo et al., 2018). Changes in cytokine and chemokine responses during AIV infection are crucial to the pathogenesis of avian influenza in chickens (Kuribayashi et al., 2013; Luo et al., 2018). With increases in infection time, some classical signaling pathways, such as the B cell receptor signaling pathway, T cell receptor signaling pathway, NF-κB signaling pathway, and other important immune-related signaling pathways, become gradually active. In addition, we found that proteasome signaling pathway was also significantly enriched in multiple groups of DEGs (0 hpi vs. 20 hpi, 12 hpi vs. 20 hpi, and 6 hpi vs. 12 hpi). Viruses confiscate cellular components of the proteasome to facilitate many aspects of the infectious cycle (van Tol et al., 2017). CCT5 was also found to interact with PA of AIV proteins and host proteins to improve our understanding of the viral life cycle, and to assist in identifying host resistance mechanisms by which they respond to AIV infection.

Several genes have been reported to be involved in viral infection. The chaperonin containing TCP1 complex (CCT) is a multi-subunit complex that can regulate protein folding (Gestaut et al., 2019). T-complex protein 1 subunit epsilon (CTCT5) interacts with RNA polymerase NS5B of the hepatitis C virus (Fislová et al., 2010). CCT5 was also found to interact with PA of H5N1 AIV, and is at the core of the virus host interaction network (Wang et al., 2016). The Ras-GTPase-activating protein SH3-domain-binding protein 1 (G3BP1) could also play an important role in the host anti-viral response through associating with RIG-I signaling. G3BP1 formed a complex with RNF125 and RIG-I, leading to decreased RNF125 expression via its auto-ubiquitination, which could inhibit the replication of Sendai virus and vesicular stomatitis virus (Yang et al., 2019). G3BP1 also synergized with RIG-I to induce the expression of the important anti-viral cytokine, IFN-β (Kim et al., 2019).

LncRNAs have been identified to regulate innate antiviral responses via different targets and in various ways, including regulating the activity of RNA binding proteins, and the translation and degradation of host mRNA (Atianand et al., 2017; Wang et al., 2017). In this study, we identified the differently expressed lncRNAs in chicken DF1 cells during H5N1 AIV infection. A total of 1,416 lncRNAs were differentially expressed, and their functions need to be further explored for a comprehensive understanding of the anti-viral mechanisms by which they respond to AIV infection.

| Term                              | Count | P-value |
|-----------------------------------|-------|---------|
| Allograft rejection               | 43    | 1.37E-15|
| Graft-versus-host disease         | 40    | 2.17E-14|
| Type I diabetes mellitus          | 42    | 3.66E-14|
| Viral myocarditis                 | 59    | 2.96E-13|
| Autoimmune thyroid disease        | 41    | 1.41E-12|
| Antigen processing and presentation | 43   | 6.97E-09|
| Natural killer cell mediated cytotoxicity | 39  | 1.12E-07|
| Cell adhesion molecules (CAMs)    | 52    | 6.65E-07|
| Phagosome                         | 60    | 7.82E-07|
| Herpes simplex infection          | 54    | 4.49E-06|
| Epstein-Barr virus infection      | 57    | 1.18E-05|
| Phenylnalalnine metabolism        | 12    | 2.94E-05|
| HTLV-I infection                  | 69    | 8.69E-05|
| Asthma                            | 12    | 0.000165336|
| Phenylalanine, tyrosine and tryptophan biosynthesis | 6  | 0.000592853|
| Fat digestion and absorption      | 14    | 0.00040793|
| Ubiquinone and other terpenoid-quinone biosynthesis | 8  | 0.001249166|
| MicroRNAs in cancer               | 39    | 0.001327914|
| Staphylococcus aureus infection   | 15    | 0.001642345|
| Phosphonate and phosphinate metabolism | 7  | 0.001645979|
| Cardiac muscle contraction        | 24    | 0.002032595|
| Leishmaniasis                     | 18    | 0.00745978|
| Tropane, piperidine and pyridine alkaloid biosynthesis | 5  | 0.003181575|
| Amyotrophic lateral sclerosis (ALS) | 14  | 0.003277039|
| Fc epsilon RI signaling pathway   | 17    | 0.0042699|

Viruses rely on host cellular functions to replicate, and thus, they hijack and rewire the host cell machinery for their own needs. We carried out research on the interactions between H5N1 AIV proteins and host proteins to improve our understanding of the viral life cycle, and to assist in identifying host resistance mechanisms. A total of 621 host proteins were obtained by affinity purification mass spectrometry (Li et al., 2016; Wang et al., 2016, 2021). By comparing the 621 host interaction proteins with the 2762 DEGs identified from the transcriptome, 129 DEGs were found among the host proteins to interact with viral proteins (Supplementary Table 7). The host proteins interacting with virus proteins and the DEGs may be the key factors affecting viral replication; thus, the overlapping genes should be focused more intently. These overlapping genes not only interacted with viral proteins, but also exhibited significant changes at the gene expression level after viral infection. Thus, those genes may be important for viral replication.
and 6,586 and 4,788 pairs of cis- and trans- acting relationships were obtained, respectively (Supplementary Tables 3, 4).

GO and pathway enrichment of the target genes of differentially expressed lncRNAs were analyzed. In terms of the biological processes identified in the GO analyses, genes associated with peptide antigen binding, regulation of T cell mediated immunity, MHC class I protein complexes, and the positive regulation of T cell-mediated immunity were significantly enriched (Supplementary Table 6). Pathway enrichment analysis revealed that 16 of the 25 significantly enriched signaling pathways were related to immunity or disease, such as antigen processing and presentation, natural killer cell mediated cytotoxicity, phagosomes, and Fc epsilon RI signaling pathways (Table 4). These results showed that lncRNAs played an important role in regulating AIV infection and/or host immunity. Collectively, these results provided a subset of lncRNAs that might play important roles in the pathogenesis of influenza viruses and add the lncRNAs to the vast repertoire of host factors used by IAV for infection and persistence.

Chrome aggregation 1 like (CSE1L) was first identified in screening tests for drug resistance in breast cancer cells. Mutations in the CSE1L homolog in yeast lead to chromosomal segregation abnormalities and mitotic block (Xiao et al., 1993). The N-terminal domain of CSE1L is homologous to the RAN binding domain of Importin β, and CSE1L is responsible for the nucleation of Importin α to realize the reuse of Importin α. The exudation and reuse of Importin α affects the exudation and transport of a variety of proteins, including host transcription factors, endogenous proteins, and invading viral proteins (Döhner et al., 2018). In the presence of RanGTP, CSE1L formed complexes with Importin-α and RanGTP and nucleated. In cytoplasm, the CSE1L/Importin-α/RanGTP complex is dissociated with the assistance of such proteins as RanBP1 and RanGAP1. Importin-α is dissociated from the complex and participates in the next round of nucleation. CSE1L can transport macromolecules out of the nucleus through importin dependent and independent pathways. For example, CSE1L can be directly responsible for the transport
found that cis-acting lncRNA MSTRG.14019.1 targeted CSE1L to the cytoplasm (Okimoto et al., 2015). In this study, we found that viral replication efficiency decreased significantly after the expression of CSE1L was reduced, indicating that CSE1L can promote the replication of H5N1 AIV. Based on the above results, we hypothesize that cis-acting lncRNA MSTRG.14019.1 down regulates the expression of CSE1L, and inhibits the replication of H5N1 AIV.

The present study is the first to characterize both the host and virus transcriptomes of H5N1-infected chicken DF1 cells at different infection time points. We revealed the trends in expression of virus and host genes, and analyzed the pathway enrichment of host DEGs. We also mapped the regulatory networks of differential lncRNAs and their target genes during viral infection, and found that the cis-acting lncRNA MSTRG.14019.1 targeted CSE1L and may affect virus replication. In conclusion, our data highlight the importance of dual RNA-seq in studying host pathogen interactions and identifying novel elements that may contribute to influenza biology and to RNA-based immune responses.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the Genome Sequence Archive repository (https://ngdc.cnbc.ac.cn/gsa/), accession number CRA006311.

AUTHOR CONTRIBUTIONS

QW, QL, and GZ conceived, designed the experiments, and wrote the manuscript. QW, ZW, JW, and GZ performed the experiments. QW and QL analyzed the data. QW, ZW, JW, MZ, and GZ contributed to reagents, materials, and analysis tools. All authors contributed to the article and approved the submitted version.

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FW, QL, and GZ conceived, designed the experiments, and wrote the manuscript. QW, ZW, JW, and GZ performed the experiments. QW and QL analyzed the data. QW, ZW, JW, MZ, JW, and GZ contributed to reagents, materials, and analysis tools. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by grants from the National Key R&D Program of China (2018YFE0128000), National Natural Science Foundation of China (Nos. 32072708 and 32102533), China Agriculture Research System of MOF and MARA (CARS-41), and the Agricultural Science and Technology Innovation Program (ASTIP-IAS04 and CAASZDRW202005).

ACKNOWLEDGMENTS

We thank Daxin Peng of the School of Veterinary Medicine, Yangzhou University for providing work space in a P3 laboratory for the virus infection experiments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.828277/full#supplementary-material

Supplementary Table 1 | Comparative analyses between the six groups of DEGs.

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