Global gene expression analysis data of chicken dendritic cells infected with H9N2 avian influenza virus

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**Abstract**

This data article reports the global gene expression analysis data of chicken DCs infected with H9N2 avian influenza virus (AIV) compared with mock infection. The differentially expressed genes (DEGs), and the data of GO enrichment analysis and KEGG pathway analysis for DEGs were reported here. In addition, some of these DEGs associated with innate immune response and antigen presentation were also verified by qPCR. The replication of H9N2 AIV in DCs, and the viability kinetic of DCs during H9N2 AIV infection, and the primers for qPCR were also reported in this data article. The data presented here was used on the research article entitled “Transcriptomic profile of chicken bone marrow-derive dendritic cells in response to H9N2 avian influenza A virus”. © 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license. (http://creativecommons.org/licenses/by/4.0/)
Specifications table

| Subject                  | Immunology and Microbiology |
|--------------------------|-----------------------------|
| Specific subject area    | The virus infection influence on the gene expression of immune cells. |
| Type of data             | Table                       |
| How data were acquired   | RNA-seq via Illumina HiSeq XTen (Illumina, USA), Real-time PCR via Thermal Cycler DICE Real-Time System Lite TP700 (Takara, Japan) |
| Data format              | Raw data, analyzed          |
| Parameters for data collection | Mock and H9N2 AIV infected bone marrow derived DCs from chickens |
| Description of data collection | Changes in the gene expression of chicken DCs for H9N2 virus infection |
| Data source location     | Key Laboratory of Veterinary Biological Engineering and Technology, Ministry of Agriculture, Nanjing, Jiangsu, China |
| Data accessibility       | Raw data of RNA Seq analysis were deposited to NCBI, and the GEO accession numbers is GSE117163. |
| Related research article | Liu Q et al., 2020, Transcriptomic profile of chicken bone marrow-derive dendritic cells in response to H9N2 avian influenza A virus, Vet Immunol Immunopathol, 220: 109,992 [1]. |

Value of the Data

- The first global gene expression analysis of chicken DCs infected with H9N2 AIV.
- These data will help to understand the host immune response to H9N2 infection in chickens.
- Expression analysis data in chicken DCs may be further used for comparative analysis with expression assays in other poultry.

1. Data description

Here we report the global gene expression analysis data of chicken DCs infected with H9N2 AIV compared with mock infection. The sequence database was deposited to NCBI, and the GEO accession numbers is GSE117163. The data show that 4151 genes were significantly up-regulated, and 2138 genes were significantly down-regulated following H9N2 AIV infection (Supplementary Table 1). GO enrichment analysis of these differentially expressed genes (DEGs) showed that a total of 130 and 120 GO terms were significantly enriched for the up- and down-regulated DEGs respectively, in three main GO categories: cellular components, molecular functions, and biological processes (Table 1, Supplementary Tables 2–4). Pathway analysis of the up-regulated and down-regulated DEGs was also performed on the KEGG database (Table 2, Supplementary Table 5). In addition, the phenotype identification of DCs, and the viability kinetic of DCs during H9N2 AIV infection, and the replication of H9N2 AIV in DCs and some of these DEGs were also determined by flow cytometric analysis and qPCR (Figs. 1 and 2), and the primers for qPCR were listed in Table 3.

2. Experimental design, materials, and methods

2.1. Cell culture and virus infection

The bone marrow (BM) monocytes were collected from femurs of four 4-week-old specific pathogen-free (SPF) white leghorn chickens, and were cultured for dendritic cells (BM-DCs) as previously described, with some modifications [2]. Briefly, BM cells were cultured in 6-well plates at a concentration of $5 \times 10^6$/ml in RPMI-1640 (Wisent) complete medium containing 5% FBS (Wisent), 100 U/ml penicillin and 100 μg/ml streptomycin for 6 h at 41 °C in 5% CO$_2$, and then non-adherent cells were removed by replacing with fresh complete medium containing 50 ng/ml chicken GM-CSF (Abcam, USA), and 10 ng/ml IL-4 (Kingfisher, USA). Half of the medium was replaced with fresh complete medium containing GM-CSF and IL-4 at day 2, 4 and 6. At day 7,
Table 1
GO terms significantly enriched by up- or down-regulated DEGs for biological process.

| GO ID     | GO Term                                         | DEGs style | -log_{10}FDR |
|-----------|-------------------------------------------------|------------|--------------|
| GO:0,006,811 | ion transport                                   | Up         | 9.526894014  |
| GO:0,055,085 | transmembrane transport                         | Up         | 8.465671499  |
| GO:0,007,186 | G-protein coupled receptor signaling pathway    | Up         | 8.129413176  |
| GO:0,007,268 | synaptic transmission                            | Up         | 7.380059989  |
| GO:0,007,267 | cell-cell signaling                              | Up         | 7.084386694  |
| GO:0,007,275 | multicellular organismal development            | Up         | 6.287296285  |
| GO:0,006,936 | muscle contraction                              | Up         | 5.973701573  |
| GO:0,034,765 | regulation of ion transmembrane transport       | Up         | 5.783910618  |
| GO:0,007,601 | visual perception                                | Up         | 5.708395794  |
| GO:0,042,391 | regulation of membrane potential                 | Up         | 5.147163012  |
| GO:0,007,155 | cell adhesion                                    | Up         | 5.131140366  |
| GO:0,030,198 | extracellular matrix organization               | Up         | 4.205396928  |
| GO:0,071,805 | potassium ion transmembrane transport           | Up         | 4.195542844  |
| GO:0,007,165 | signal transduction                              | Up         | 3.76675983   |
| GO:0,006,813 | potassium ion transport                          | Up         | 3.708036028  |
| GO:0,034,220 | ion transmembrane transport                     | Up         | 3.605281638  |
| GO:0,042,472 | inner ear morphogenesis                          | Up         | 3.271156529  |
| GO:0,010,951 | negative regulation of endopeptidase activity    | Up         | 3.191259258  |
| GO:0,007,602 | phototransduction                                | Up         | 2.683349047  |
| GO:0,007,218 | neuroptide signaling pathway                    | Up         | 2.657366515  |
| GO:0,007,154 | cell communication                               | Up         | 2.621742773  |
| GO:0,006,814 | sodium ion transport                             | Up         | 2.552679391  |
| GO:0,030,818 | negative regulation of cAMP biosynthetic process| Up         | 2.305595239  |
| GO:0,007,605 | sensory perception of sound                     | Up         | 2.287929054  |
| GO:0,007,166 | cell surface receptor signaling pathway          | Up         | 2.176002285  |
| GO:0,050,953 | sensory perception of light stimulus            | Up         | 1.856059697  |
| GO:0,006,812 | cation transport                                 | Up         | 1.836705255  |
| GO:0,051,216 | cartilage development                           | Up         | 1.752329879  |
| GO:0,030,049 | muscle filament sliding                          | Up         | 1.750389712  |
| GO:0,019,229 | regulation of vasoconstriction                  | Up         | 1.750389712  |
| GO:0,001,974 | blood vessel remodeling                         | Up         | 1.711364263  |
| GO:0,007,187 | G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger | Up | 1.698685935 |
| GO:0,070,588 | calcium ion transmembrane transport             | Up         | 1.570097419  |
| GO:0,009,607 | response to biotic stimulus                     | Up         | 1.561075333  |
| GO:0,009,612 | response to mechanical stimulus                 | Up         | 1.539448937  |
| GO:0,008,272 | sulfate transport                                | Up         | 1.527518462  |
| GO:1,902,358 | sulfate transmembrane transport                 | Up         | 1.458583631  |
| GO:0,030,154 | cell differentiation                            | Up         | 1.436427558  |
| GO:0,019,532 | oxalate transport                               | Up         | 1.433603081  |
| GO:0,007,204 | positive regulation of cytosolic calcium ion concentration | Up | 1.419354807 |
| GO:0,015,701 | bicarbonate transport                           | Up         | 1.404425818  |
| GO:0,035,725 | sodium ion transmembrane transport              | Up         | 1.386341211  |
| GO:0,070,098 | chemokine-mediated signaling pathway            | Up         | 1.359987939  |
| GO:0,009,653 | anatomical structure morphogenesis              | Up         | 1.344405489  |
| GO:0,002,027 | regulation of heart rate                        | Up         | 1.339913059  |
| GO:0,050,896 | response to stimulus                            | Up         | 1.323489649  |
| GO:0,031,018 | endocrine pancreas development                  | Up         | 1.306589409  |
| GO:0,008,152 | metabolic process                               | Down       | 13.17523377  |
| GO:0,000,278 | mitotic cell cycle                              | Down       | 11.09472543  |

(continued on next page)
| GO ID     | GO Term                                                                 | DEGs style | -log_{10}FDR |
|-----------|-------------------------------------------------------------------------|------------|--------------|
| GO:0,044,281 | small molecule metabolic process                                        | Down       | 6.206417925  |
| GO:0,055,114 | oxidation-reduction process                                             | Down       | 4.690526235  |
| GO:0,042,590 | antigen processing and presentation of exogenous peptide antigen via MHC class I | Down       | 3.526556283  |
| GO:0,002,474 | antigen processing and presentation of peptide antigen via MHC class I | Down       | 3.506546589  |
| GO:0,031,145 | anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process | Down       | 3.506546589  |
| GO:0,005,975 | carbohydrate metabolic process                                          | Down       | 3.26355729   |
| GO:0,006,260 | DNA replication                                                         | Down       | 3.152057253  |
| GO:0,000,082 | G1/S transition of mitotic cell cycle                                  | Down       | 3.114065771  |
| GO:0,044,255 | cellular lipid metabolic process                                       | Down       | 3.064571406  |
| GO:0,051,439 | regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle | Down       | 3.064571406  |
| GO:0,006,457 | protein folding                                                        | Down       | 2.82243026   |
| GO:0,006,418 | tRNA aminoacylation for protein translation                             | Down       | 2.776822875  |
| GO:0,007,094 | mitotic spindle assembly checkpoint                                     | Down       | 2.776822875  |
| GO:0,007,067 | mitotic nuclear division                                               | Down       | 2.768732327  |
| GO:0,002,479 | antigen processing and presentation of exogenous peptide antigen via MHC class I | Down       | 2.69855525   |
| GO:0,006,200 | ATP catabolic process                                                  | Down       | 2.602969703  |
| GO:0,007,049 | cell cycle                                                             | Down       | 2.524100463  |
| GO:0,034,976 | response to endoplasmic reticulum stress                               | Down       | 2.444228149  |
| GO:0,006,271 | DNA strand elongation involved in DNA replication                      | Down       | 2.444228149  |
| GO:0,007,076 | mitotic chromosome condensation                                        | Down       | 2.34198832   |
| GO:0,043,277 | apoptotic cell clearance                                               | Down       | 2.34198832   |
| GO:0,015,031 | protein transport                                                      | Down       | 2.315189616  |
| GO:0,006,270 | DNA replication initiation                                             | Down       | 2.273274234  |
| GO:0,006,629 | lipid metabolic process                                                | Down       | 2.26057764   |
| GO:0,051,437 | positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle | Down       | 2.14441609   |
| GO:0,051,436 | negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle | Down       | 2.14441609   |
| GO:0,030,968 | endoplasmic reticulum unfolded protein response                         | Down       | 2.130960444  |
| GO:0,030,261 | chromosome condensation                                                | Down       | 2.094490573  |
| GO:0,044,267 | cellular protein metabolic process                                      | Down       | 2.083055589  |
| GO:0,043,687 | post-translational protein modification                                | Down       | 2.013949561  |
| GO:0,006,099 | tricarboxylic acid cycle                                               | Down       | 1.953559525  |
| GO:0,006,508 | proteolysis                                                            | Down       | 1.866941451  |
| GO:0,006,509 | membrane protein ectodomain proteolysis                                | Down       | 1.781469722  |
| GO:0,051,301 | cell division                                                          | Down       | 1.751025857  |
| GO:0,006,635 | fatty acid beta-oxidation                                              | Down       | 1.657618606  |
| GO:0,032,201 | telomere maintenance via semi-conservative replication                 | Down       | 1.51911888   |
| GO:0,033,540 | fatty acid beta-oxidation using acyl-CoA oxidase                       | Down       | 1.51911888   |
| GO:0,019,885 | antigen processing and presentation of endogenous peptide antigen via MHC class I | Down       | 1.51911888   |
| GO:0,006,665 | sphingolipid metabolic process                                         | Down       | 1.444993539  |
| GO:0,090,382 | phagosome maturation                                                   | Down       | 1.376321186  |
| GO:0,006,687 | glycosphingolipid metabolic process                                    | Down       | 1.36012919   |
| GO:0,051,701 | interaction with host                                                  | Down       | 1.36012919   |
| GO:0,030,433 | ER-associated ubiquitin-dependent protein catabolic process            | Down       | 1.313495682  |
the surface markers of BM-DCs were analyzed by flow cytometry with antibodies as previous experiments [3], and then BM-DCs were used for the infection of H9N2 AIV.

Three H9N2 subtype avian influenza viruses, A/duck/Nanjing/06/2003(NJ06), A/chicken/Changzhou/0504/2017(CZ0504), and A/chicken/Anhui/S02/2013(AHS02), were propagated in SPF white leghorn chicken eggs respectively, and the allantoic fluid was concentrated via sucrose gradient ultracentrifugation and resuspended in RPMI. The allantoic fluid from mock infected eggs was processed in the same manner and used for mock infection. Viral titers were measured by calculating the 50% tissue culture infectious dose (TCID\textsubscript{50}) in MDCK cells. Unless otherwise stated in the text, H9N2 AIV refers to NJ06. BM-DCs (2 × 10\textsuperscript{6}/ml) were infected with H9N2 AIV (10\textsuperscript{6} TCID\textsubscript{50}/0.1 ml) and then were collected for RNA sequencing at 6 h post infection. Three independent biological replicates of the cell culture and virus infection experiments were performed for the RNA sequencing analysis.

### 2.2. RNA sequencing

Total RNA was extracted from BM-DCs using TRIzol reagent (Invitrogen). The integrity and concentration of the extracted RNA were assessed by Agilent 2200 Bioanalyzer (Agilent

| Pathway ID | Pathway Term | DEGs style | -log\textsubscript{10}FDR |
|------------|--------------|-------------|--------------------------|
| PATH:04,080 | Neuroactive ligand-receptor interaction | Up | 9.228962756 |
| PATH:04,060 | Cytokine-cytokine receptor interaction | Up | 7.94406601 |
| PATH:04,512 | ECM-receptor interaction | Up | 3.380229513 |
| PATH:04,610 | Complement and coagulation cascades | Up | 2.860948 |
| PATH:04,744 | Phototransduction | Up | 2.636047378 |
| PATH:04,974 | Protein digestion and absorption | Up | 2.323949981 |
| PATH:04,950 | Maturity onset diabetes of the young | Up | 2.323949981 |
| PATH:04,975 | Fat digestion and absorption | Up | 1.709952575 |
| PATH:00,830 | Retinol metabolism | Up | 1.709952575 |
| PATH:04,350 | TGF-beta signaling pathway | Up | 1.532818581 |
| PATH:04,151 | PI3K-Akt signaling pathway | Up | 1.458023559 |
| PATH:04,020 | Calcium signaling pathway | Up | 1.458023559 |
| PATH:04,724 | Glutamatergic synapse | Up | 1.458023559 |
| PATH:05,217 | Basal cell carcinoma | Up | 1.458023559 |
| PATH:04,978 | Mineral absorption | Up | 1.458023559 |
|(PATH:05,033 | Nicotine addiction | Up | 1.458023559 |
| PATH:00,591 | Linoleic acid metabolism | Up | 1.32641377 |
| PATH:04,142 | Lysosome | Down | 9.91673 |
| PATH:01,100 | Metabolic pathways | Down | 6.428568 |
| PATH:01,200 | Carbon metabolism | Down | 5.71436 |
| PATH:04,145 | Phagosome | Down | 5.411231 |
| PATH:04,141 | Protein processing in endoplasmic reticulum | Down | 4.305033 |
| PATH:00,970 | Aminoacyl-tRNA biosynthesis | Down | 4.021603 |
| PATH:03,030 | DNA replication | Down | 3.303353 |
| PATH:00,020 | Citrate cycle (TCA cycle) | Down | 2.92431 |
| PATH:00,640 | Propanoate metabolism | Down | 2.92431 |
| PATH:04,110 | Cell cycle | Down | 2.37307 |
| PATH:03,430 | Mismatch repair | Down | 2.300895 |
| PATH:03,410 | Base excision repair | Down | 2.228318 |
| PATH:00,531 | Glycosaminoglycan degradation | Down | 1.872496 |
| PATH:00,071 | Fatty acid degradation | Down | 1.833489 |
| PATH:01,230 | Biosynthesis of amino acids | Down | 1.782571 |
| PATH:00,030 | Pentose phosphate pathway | Down | 1.497989 |
| PATH:00,860 | Porphyrin and chlorophyll metabolism | Down | 1.397797 |
| PATH:01,212 | Fatty acid metabolism | Down | 1.325871 |
| PATH:01,210 | 2-Oxocarboxylic acid metabolism | Down | 1.325871 |
Table 3
Primer names used for qPCR.

| Primer name | Sequence(5′−3′) | GenBank accession no. |
|-------------|-----------------|-----------------------|
| CCL4-F      | CTCATCCAGAGGCACCTACA | NM_204,720.1 |
| CCL4-R      | GCCCTGAGCTTGAGCAGTGA | NM_204,438.2 |
| CCL20-F     | TTGCTGTTTGGGAAGTGTGATTA | NM_204,438.2 |
| CCL20-R     | AAGGATTGACAGCGCTTCCA | NM_204,438.2 |
| CCL28-F     | GGGTTTACTGTTGACAGAG | XM_015272682 |
| CCL28-R     | ATCCGGTGCTTACGCTACAGA | NM_204,510.1 |
| CXCL12-F    | CTTGGAAGTGAAGATCAGTGTC | NM_204,510.1 |
| CXCL12-R    | AATGCTGAAAGAGCGTTGGA | NM_204,510.1 |
| CXCL11-F    | CCGACCATCTGCAAGAAATG | NM_205,018.1 |
| CXCL11-R    | GCCCTGCAAGATTTGCTTTC | NM_205,498.1 |
| CXCL2-F     | CTGCGGTCAGCAGTCATTAG | NM_205,498.1 |
| CXCL2-R     | AGGAGACACCTTCTTCCATCC | NM_205,498.1 |
| IL-1β-F     | TTGCTCTGCTGAGTGCACAC | HQ329098.1 |
| IL-1β-R     | GCCATCGACCCAGTGTTCA | HM179640.1 |
| IL-6-F      | GCTGGACATTGACAGCTGAG | NM_204,720.1 |
| IL-6-R      | TGGGCTTGAGAAGACCAAG | AY262751.1 |
| IL-12A-F    | AATCTCTTTTCCAGGCTCACTCA | NM_204,720.1 |
| IL-12A-R    | AAGTTGCGGAAACAATACCTG | NM_204,720.1 |
| IL-12B-F    | CATTCTGCACTTGGAGTTCAT | NM_204,720.1 |
| IL-12B-R    | CAGGCTCTGACGCTTTCAT | JQ776598.1 |
| IL-17-F     | CTAGTTGCTGAGTCTTTC | NM_204,720.1 |
| IL-17-R     | AAGCTTCCAGGCAACTAT | AJ617782.1 |
| IL-22-F     | CAGACTCTTGCTGAGTTC | NM_204,720.1 |
| IL-22-R     | GTAGCTCTCTCCCTGCTCTC | NM_204,720.1 |
| IRF7-F      | AGTTGCTGCTGAGTCTTTC | NM_204,720.1 |
| IRF7-R      | AGTTGCTGCTGAGTCTTTC | NM_204,720.1 |
| IFIH1-F     | ATCCGAGAATCCACAGTTC | NM_204,720.1 |
| IFIH1-R     | AACTCTTCTCTTGGAGTTC | NM_204,720.1 |
| IFN-β-F     | CTGGCCCAACAAGGCTG | AJ974089.1 |
| IFN-β-R     | CTGGCCCAACAAGGCTG | AJ974089.1 |
| IFN-κ-F     | GAGAAATTGACGGCCGCTCAT | KR817821.1 |
| IFN-κ-R     | CATTCTTGACGGCTGATCT | KR817821.1 |
| OASL-F      | GCTGTCAGCTGAACTGCTTGC | NM_205,041.1 |
| OASL-R      | CTTTTAGCTTACGCTGAGG | NM_205,041.1 |
| RSAD2-F     | TGCTCAAGAGAAGGAAACG | NM_001318443.1 |
| RSAD2-R     | TGATTAGCCTGAAACG | NM_001318443.1 |
| IFIT5-F     | TTGCTCCACGCTGTGTCATG | KT180229.1 |
| IFIT5-R     | TGCGCTTGGACGGCTCACTTG | KT180229.1 |
| IFN-γ-F     | TGGCCCAACAAGGCTG | AJ974089.1 |
| IFN-γ-R     | TGGCCCAACAAGGCTG | AJ974089.1 |
| TLR3-F      | CCAACACTTCTGGAATACGCTTGC | MF576162.1 |
| TLR3-R      | TTACTGATATAGCCGGAACAGATTTCC | MF576162.1 |
| DDX60-F     | ACCGGCTTGCTGTTTGGTAGA | XM_004940918.3 |
| DDX60-R     | TCCAAAACCTCTGCTCACAAT | MF563593.1 |
| DHX58-F     | AGGCCAACAGAAGCTGACGA | MF563593.1 |
| DHX58-R     | CGCGAACATCGCCGACTTCT | MF563593.1 |
| IFITM3-F    | ATCGGACCTTGGAGGCTTGGT | NM_001350061.1 |
| IFITM3-R    | TGCGTCTGGGCTGTAAGAA | NM_001350061.1 |
| PSMB7-F     | AAGGAGCACCTGGGAGG | NM_001350061.1 |
| PSMB7-R     | AAGGAGCACCTGGGAGG | NM_001350061.1 |

(continued on next page)
Table 3 (continued)

| Primer name | Sequence(5′−3′) | GenBank accession no. |
|-------------|-----------------|-----------------------|
| PSMC1-F     | GAGGGAGATCCAGCGTACAA | NM_204,958.1 |
| PSMC1-R     | GTCGATACGTCCTGGCCTAA | NM_001006225.1 |
| PSMC2-F     | GCTGGTGCAAGATATTGTG | NM_00103190.1 |
| PSMC2-R     | ACCATCATCAAGCCAGCAC | NM_001012934.1 |
| PSMC3-F     | CAGGAGGAGATCCAGCGTACAA | NM_00103190.1 |
| PSMC3-R     | ACCCATCATCAAGCCAGCAC | NM_001012934.1 |
| PSMD2-F     | GCTGGTGCAGAAGTATGTGG | NM_001031362.1 |
| PSMD2-R     | ACATGAGGGATCCAGCGTACAA | NM_00103190.1 |
| PSMD3-F     | GCTGGTGCAGAAGTATGTGG | NM_001031362.1 |
| PSMD3-R     | GACTCTGCGAGGAACACAAC | NM_001031362.1 |
| TPP2-F      | CACTCTGCGAGGAACACAAC | NM_001031362.1 |
| TPP2-R      | NM_205,283.2 |
| LAMP1-F     | NM_205,283.2 |
| LAMP1-R     | NM_205,283.2 |
| M6PR-F      | NM_205,283.2 |
| M6PR-R      | NM_205,283.2 |
| TCIRG1-F    | NM_205,283.2 |
| TCIRG1-R    | NM_205,283.2 |
| CTSB-F      | NM_205,283.2 |
| CTSB-R      | NM_205,283.2 |
| CTSS-F      | NM_205,283.2 |
| CTSS-R      | NM_205,283.2 |
| CD74-F      | NM_205,283.2 |
| CD74-R      | NM_205,283.2 |
| DCTN1-F     | NM_205,283.2 |
| DCTN1-R     | NM_205,283.2 |
| IFI30-F     | NM_205,283.2 |
| IFI30-R     | NM_205,283.2 |
| LGMN-F      | NM_205,283.2 |
| LGMN-R      | NM_205,283.2 |
| RAGAP1-F    | NM_205,283.2 |
| RAGAP1-R    | NM_205,283.2 |
| UNC93B1-F   | NM_205,283.2 |
| UNC93B1-R   | NM_205,283.2 |
| H9N2 AIV M1-F | NM_205,283.2 |
| H9N2 AIV M1-R | NM_205,283.2 |
| Actinb-F    | NM_205,283.2 |
| Actinb-R    | NM_205,283.2 |

Technologies, USA). RNA samples with RNA Integrity Number ≥ 7 were used for library construction. The libraries were prepared using the TruSeq RNA Sample Preparation Kit (Illumina, USA) according to the manufacturer’s protocol, and then sequenced on the Illumina HiSeq XTen (Illumina, USA). The construction and sequencing of libraries were performed by Shanghai Bioinformatics (Shanghai, China), and the GEO accession numbers for the RNA-seq data is GSE117163.

2.3. Data analysis

The raw reads were filtered by removing the adaptor sequences and low-quality reads containing more than 5% ambiguous bases (noted as N) or more than 20% of bases with qualities of <20 to obtain clean reads. Thereafter, the clean reads were mapped to the Chicken genome (Version: Gallus_gallus-5.0 NCBI), using HISAT2 with default parameter. The gene expression data were generated and normalized by fragments per kilobase of transcript per million uniquely mapped reads (FPKM) [4]. Differentially expressed genes (DEGs) analysis was performed using
Fig. 1. Phenotype identification and H9N2 AIV infection of BM-DCs. The surface molecule MHC Class II (A) and CD11c (B) on the BM-DCs were determined by flow cytometric analysis using mouse anti-human CD11c antibody (eBioscience, USA) or mouse anti-chicken MHCII antibody (Abcam, USA). (C) BM-DCs were infected with the H9N2 AIV, and the total RNA was isolated from H9N2 AIV infected BM-DCs for analysis of the viral M1-specific RNAs by SYBR Green real-time PCR. The expression levels of viral M1 gene at 3, 6, 18 h post infection are presented as the relative gene expression in relation to that at 0.5 h post infection, which represent the increases of viral RNA levels during the time course of infection. The results are presented as means from triplicate measurements with standard deviations. (D) H9N2 AIV and mock infected BM-DCs were collected at 3, 6, 18 h and stained with Annexin V for flow cytometric analysis. Data on percentage of living cells is presented as mean values from triplicate measurements with standard deviations.

DEGSeq algorithm, and DEGs with a p-value <0.05, a FDR <0.05 and a fold change >2 were selected for GO and KEGG pathway enrichment analyses, respectively. The GO and KEGG pathways were considered significantly enriched when FDR < 0.05.

2.4. Quantitative real-time PCR

The DEGs recognized by RNA-seq was verified by Quantitative real-time PCR (qPCR). Total RNA was isolated from a replica RNA sequencing infection experiment using TRizol reagent (Life Technologies) and treated with DNase I (Fermentas, Glen Burnie, MD, USA). One microgram of total RNA per sample was reverse transcribed into cDNA using a PrimeScript RT Reagent Kit (Takara). The qPCR was performed using Talent qPCR PreMix SYBR Green (Tiangen, China) on a Real-Time System Lite TP700 (Takara, Japan). The product specificity of qPCR was verified by one cycle for melting curve analysis. The expression of each cytokine gene relative to that of the β-actin was calculated using the $2^{-\Delta\Delta CT}$ method. All primers for these target genes are listed in Table 3.
Fig. 2. BM-DCs were infected with A/duck/Nanjing/06/2003(NJ06), A/chicken/Changzhou/0504/2017(CZ0504), and A/chicken/Anhui/S02/2013(AHS02) H9N2 AIV strains and were collected at 6 h post infection. (A) The total RNA was isolated from BM-DCs for analysis of the viral M1-specific RNAs by SYBR Green real-time PCR and the expression levels of viral M1 gene are presented as the relative gene expression in relation to that at 0.5 h post infection. The results are presented as means from triplicate measurements with standard deviations. (B) H9N2 AIV and mock infected BM-DCs were collected and stained with Annexin V for flow cytometric analysis. Data on percentage of living cells is presented as mean values from triplicate measurements with standard deviations. (C and D) The DEGs involved in host innate immune responses and antigen presentation were selected to be confirmed by qPCR. The histograms indicate the qPCR data which are expressed as the means standard deviations (SD) for triplicate infections.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have influenced the work reported in this article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105430.
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