Identification and antiviral effect of Cherry Valley duck IRF4

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ABSTRACT Interferon regulatory factor 4 (**IRF4**) is a multifunctional transcription factor that plays an important regulatory role in the interferon (**IFN**) signaling. IRF4 participates in the process of antivirus, Th cell differentiation and B cell maturation by regulating the expression of IFN and some lymphokines. In this study, Cherry Valley duck IRF4 (**duIRF4**) was cloned and its cDNA was analyzed. Expression of duIRF4 in a wide variety of tissues and changes in duIRF4 expression due to viral infection also was detected by quantitative real-time PCR. The results show that duIRF4 contains 1,341 bp of ORF encoding a protein with 446 amino acids and contains 3 domains: DNA-binding domain (**DBD**), IRF-association domain (**IAD**) and nuclear localization signal (**NLS**). Quantitative real-time PCR analysis showed that duIRF4 was evenly expressed in all tissues examined, with the highest expression in the spleen, followed by the bursa of Fabricius, and lower in the skin and brain. In addition, expression of duIRF4 in the brain and spleen was significantly upregulated after being infected by duck plague virus, duck Tembusu virus, and novel duck reovirus. These data suggest that duIRF4 may be involved in innate immune response.

Key words: IRF4, cloning, Cherry Valley duck, innate immunity

2022 Poultry Science 101:101560
https://doi.org/10.1016/j.psj.2021.101560

INTRODUCTION

Interferon regulatory factors (**IRFs**) are a family of transcription factors that play an important role in the regulation of interferon (**IFN**) expression. The research on the family of IRFs can be traced back to 1988. The study found that a mouse nuclear factor specifically binds to the upstream regulatory region of the IFN-β gene and mediates the transcription of the IFN-β (Miyamoto et al., 1988). The IRF family includes 9 members (IRF1-IRF9), which play a regulatory function in the immune system and tumorigenesis and are widely expressed in various tissues. At the same time, many IRF members play a central role in the regulation of cell differentiation and gene expression of hematopoietic cells (Tamura et al., 2008). The IRF family is composed of a single polypeptide chain, and all have an N-terminal DNA binding domain (**DBD**). DBD can bind to the DNA sequence of the IFN stimulus response element (**ISRE**) (Darnell et al., 1994) to form a helix-helix-helix structure. In addition, the carboxyl-terminal region contains a conserved IRF association domain (**IAD**) 1 or IAD2, which mediates the intermolecular interaction of homodimers and heterodimers with other IRF members, transcription factors and cofactors. As a member of the IRF family, IRF4 is only locally expressed in immune cells such as T and B lymphocytes, macrophages, and dendritic cells. This is related to the regulation of the differentiation of these immune cells. It is considered to be the essential transcription factor for immune responses such as lymphocyte activation and immunoglobulin production (Nam and Lim, 2016). The gene regulation effect of IRF4 is mainly derived from the interaction with different transcription factors to play an immune activation or immunosuppressive effect (Biswas et al., 2010). Take for example IRF4 acts as a transactivator of the immunoglobulin light chain enhancer by cooperating with ETS protein (Eisenbeis et al., 1995). IRF4 regulates

Received August 7, 2021.
Accepted October 10, 2021.

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the expression of IFN and some lymphokines through the transcription factor complex formed by the functional domain of the PEST region and the transcription factor PU.1 (Brass et al., 1999). However, other IRF is connected to other transcription factors through the IAD. In fibroblasts, the expression of interleukin 1 (**IL-1**) is activated by the combination of IRF4 and PU.1, while the binding capacity of IRF4 and PU.1 is increased by IRF1 and IRF2 (Marecki et al., 2001). It is worth noting that when pathogens invade the body, IRF4 participates in innate immunity and adaptive immunity, which is mainly related to its regulation of the development of B cells and T cells. Studies have shown that IRF4 is a key transcriptional regulator of B cell development and function (Pathak et al., 2011). B cells that produce high-affinity antibodies in humoral immunity are destined during proliferation either differentiate into plasma cells or become memory B cells, and plasma cells are long-lived mediators of lasting humoral immunity. Studies have shown that the germinal center B cells of transgenic mice that have conditionally deleted IRF4 lack the germinal center postplasma cells and cannot differentiate memory B cells into plasma cells (Klein et al., 2006). This indicates that the production and differentiation of plasma cells require the transcription factor IRF4, and IRF4 has an independent function in this process. In B cells, IRF4 is necessary for regulating the differentiation of B cells into antibody-secreting plasma cells and germinal center cells. In T cells, IRF4 is required for the differentiation of $CD4^+$ T cell subsets, functional regulatory T cells, and effector and congenital $CD8^+$ T cells (Lohoff et al., 2002; Rengarajan et al., 2002; Brustle et al., 2007; Staudt et al., 2010; Mittrucker et al., 2017). In response to acute viral infections, $CD8^+$ T cells differentiate and expand into effector cells and stable memory cell pools. After the acute viral infection is cleared, the population shrinks and only a small portion of high-efficiency memory T cells survive. Studies have shown that IRF4 controls the response of $CD8^+$ T cells to acute viral infection in a dose-dependent manner by correlating T cell receptors (**TCR**) signals with the transcription of $CD8^+$ T cells (Nayar et al., 2014). At the same time, IRF4 also affects the number of short-lived effector cells. In the process of chronic viral stimulation, $CD8^+$ T cells acquire an exhausted phenotype, the purpose of which is to prevent excessive immune pathology. However, T cell exhaustion during chronic viral infection is driven by the transcription factor IRF4 induced by a large number of TCR (Man et al., 2017). A large amount of IRF4 also restricts the development of memory-like T cells, which can supplement effector T cells under continuous antigen stimulation. In general, whether it is acute or chronic viral stimulation, IRF4 plays a vital role in it.

Cherry Valley ducks are widely farmed in China and have created huge economic benefits. However, in recent years, the outbreak of duck plague virus (**DPV**), duck Tembusu virus (**DTMUV**), novel duck reovirus (**NDRV**), and other virus diseases has caused great economic losses to duck industry. DTMUV is an enveloped, positive-sense, single-stranded RNA virus (Su et al., 2011). Spleen enlargement, necrosis, "marble-like", and neurological symptoms are the main features. DPV is an enveloped double-stranded DNA virus (Niu et al., 2021). Duck Plague (**DP**), also known as Duck Virus Enteritis, is an acute, febrile, contagious and septic disease of waterfowl (duck, goose, and swan), characterized by liver necrosis, hemorrhage, and high mortality. NDRV is a non-enveloped, double-stranded RNA virus that normally causes liver and spleen necrosis (Zhu et al., 2015). At present, the biological functions of interferon regulatory factors are mostly studied on mammals, most of which are reported on mice, chickens, and fish (Xu et al., 2014). However, there are relatively few reports on waterfowl, and research on interferon regulatory factors on ducks has not yet appeared. Understanding the antiviral response of the Cherry Valley duck will help control the viral diseases of this species with important commercial value. Here, we report the structure of duIRF8 and the changes in the expression level of IRF4 after virus infection. The results show that duIRF8 is involved in the antiviral response of Cherry Valley duck.

MATERIALS AND METHODS

Animals and Virus Strains

The 1-day-old Cherry Valley duck was purchased from a duck embryo hatchery in Tai'an and raised in an isolator to allow the animals to eat and drink normally. These ducks were raised for 3 wk to adapt to their living environment. All duck serum samples used in the study was tested by ELISA and qRT-PCR to ensure that they were all negative for DTMUV, DPV, and NDRV before infection of animals. DTMUV, DPV and NDRV used in this experiment have been mentioned in previous studies (Yan et al., 2011; Li et al., 2016; Li et al., 2018).

Animal Experiment Design and Organization Acquisition

In order to evaluate the expression of IRF4 in normal tissues, 3 healthy ducks were euthanized, and 21 tissues were collected including heart, liver, spleen, lung, kidney, brain, cerebellum, brainstem, thymus, pancreas, bursa of fabric, duodenum, jejunum, ileum, cecum, skin, muscle, glandular stomach, muscular stomach, trachea, and esophagus. Store all tissues at −80°C for subsequent RNA extraction.

Three-wk-old ducks were randomly divided into 4 groups with 30 ducks per group. Each duck in group A was injected intramuscularly with DTMUV (0.4 mL $10^{5.2}$ $TCID_{50}$/mL per duck), each duck in group B was injected intramuscularly with DPV (0.3 mL $10^{6.5}$ $TCID_{50}$/mL per duck), each duck in group C was injected intramuscularly with NDRV (0.5 mL $10^{4.5}$ $TCID_{50}$/mL per duck), group D was the control group

for each injects 0.4 mL of sterile phosphate buffer. Three random ducks from each group were euthanized, and their spleens and brains were dissected for RNA extraction at 1, 3, 5 dpi. At the end of the study, the remaining ducks were euthanized by intravenous injection of sodium pentobarbital.

Cloning and Structure Analysis of duIRF4

The primers of duIRF4 are designed according to the predicted sequence of the National Center for Biotechnology Information (**NCBI**). Total RNA was extracted using Trizol reagent from tissues. The duCD4 gene was amplified by RT-PCR from cDNA using HiScript II One-Step RT-PCR kit for qPCR (R223-01, Vazyme, Nanjing, China). PCR products were subjected to 1% agarose gel electrophoresis. The PCR products after electrophoresis were identified on an agarose gel under ultraviolet light and were purified by using the agarose gel DNA fragment recovery kit (DP209-03, TIANGEN, Beijing, China). Finally, PCR amplified fragments were sequenced. Sequences were processed and edited in the software DNASTAR, and the full-length sequence of the IRF4 gene was obtained. Full length of IRF4 gene was analyzed by the BLAST tool at NCBI server. The phylogenetic relationship of IRF4 was established using the neighbor-joining algorithm in MEGA5.1, and GenBank accession numbers of the reference sequences used in the phylogenetic analysis are shown in Table 2.

The Expression of IRF4 in Tissues

We used 1 μg RNA to reverse transcription by the above method. The expression of duIRF4 gene in various tissues was analyzed by qRT-PCR. QRT-PCR was used to assay duIRF4 expression relative to β-actin expression, and all samples were run in 3 replicates. The reaction conditions were pre-denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 10 s, annealing at 60°C for 34 s. Finally, the dissolution curve is added. In addition, signals were detected by an ABI 7500 Real-Time PCR System. Obtain the standard curve and threshold cycles (**Ct**) value according to qRT-PCR ($15<Ct<35$). The $2^{-\Delta\Delta Ct}$ method was used to determine the relative quantitative gene expression levels with β-actin as a reference. The relative expression levels of IRF4 in 21 tissues of healthy ducks, as well as the spleen and brain of the experimental group and the control group were calculated.

Data Analysis

All values are represented as mean ± SD of triplicate samples, and analyzed by using SPSS19.0 software and performed using graph prism 5.0 software. Students' t test were used to analyze the difference between 2 groups. One-way ANOVA was used to analyze data with multiple groups.

Table 1. Primer sequences used in this study.

Primer name	Nucleotide sequence (5'-3')	Purpose
duIRF4-F	TGAGTGGCATGAACTTGGAG	Gene cloning
duIRF4-R	AACCCTTGATTCCCCTGAAC	
q-duIRF4-F	GCGGGGAGTAATACTGTGGA	qRT-PCR
q-duIRF4-R	AACCTGGTATCTGGGCAGTG	
q-β-actin-F	GGTATCGGCAGCAGTCTTA	qRT-PCR
q-β-actin-R	TTCACAGAGGCGAGTAACTT	

Note: F, Forward primer; R, reverse primer; q, qRT-PCR.

RESULTS

Cloning and Structure Analysis of DuIRF4

The duIRF4 gene is amplified by using the cDNA as template and 2 specific primers (Table 1). The duIRF4 sequence contains an ORF of 1,341 bp, which encodes 446 amino acids (GenBank: MZ683402). In addition, we can also get that the duIRF4 can be divided into 7 regions: N terminus, DNA-binding domain, putative nuclear localization signal, transactivation domain, exon 6, IRF-association domain, and C terminus (Figure 1).

Sequence Comparison and Phylogenetic Analysis of duIRF4

In order to further study the duIRF4, we compare the duIRF4 with mammals, birds, reptiles, and fish to construct a phylogenetic tree (Figure 2A). The results show that the Cherry Valley duck has the highest homology with *Anser cygnoides*, with a homology of 98.2% (Figure 2B). In addition, the duIRF4 has a very high homology with *Meleagris gallopavo* and *Chelonia mydas*, but it is relatively distantly related to fish.

Tissue Distribution of duIRF4

In order to study the distribution of IRF4 in the tissues of healthy Cherry Valley ducks, we extracted tissue RNA from the heart, liver, spleen, lungs, kidneys, brain, cerebellum, brainstem, thymus, pancreas, bursa of fabric, duodenum, jejunum, ileum, cecum, skin, muscle, glandular stomach, muscular stomach, trachea, and esophagus of healthy ducks. IRF4 mRNA expression in

Table 2. Reference sequences information of IRF4.

Species	GeneBank accession numbers
Alligator Sinensis	XP_006022269.1_1
Anser cygnoides	XP_013049051.1_1
Bos taurus	XP_005223912.1_1
Callorhinchus milii	XP_007887806.1_1
Cavia porcellus	XP_013000546.1_1
Felis catus	XP_006931571.1_1
Homo	XP_006715153.1_1
Leptonychotes	XP_006734466.1_1
Meleagris gallopavo	XP_010706716.1_1
Mus musculus	XP_036013752.1_1
Oreochromis niloticus	XP_003437930.1_1
Ovis aries	XP_027814722.1_1
Sus scrofa	XP_020953623.1_1
Chelonia mydas	XP_007058888.1_1
Pelodiscus sinensis	XP_014436930.1_1

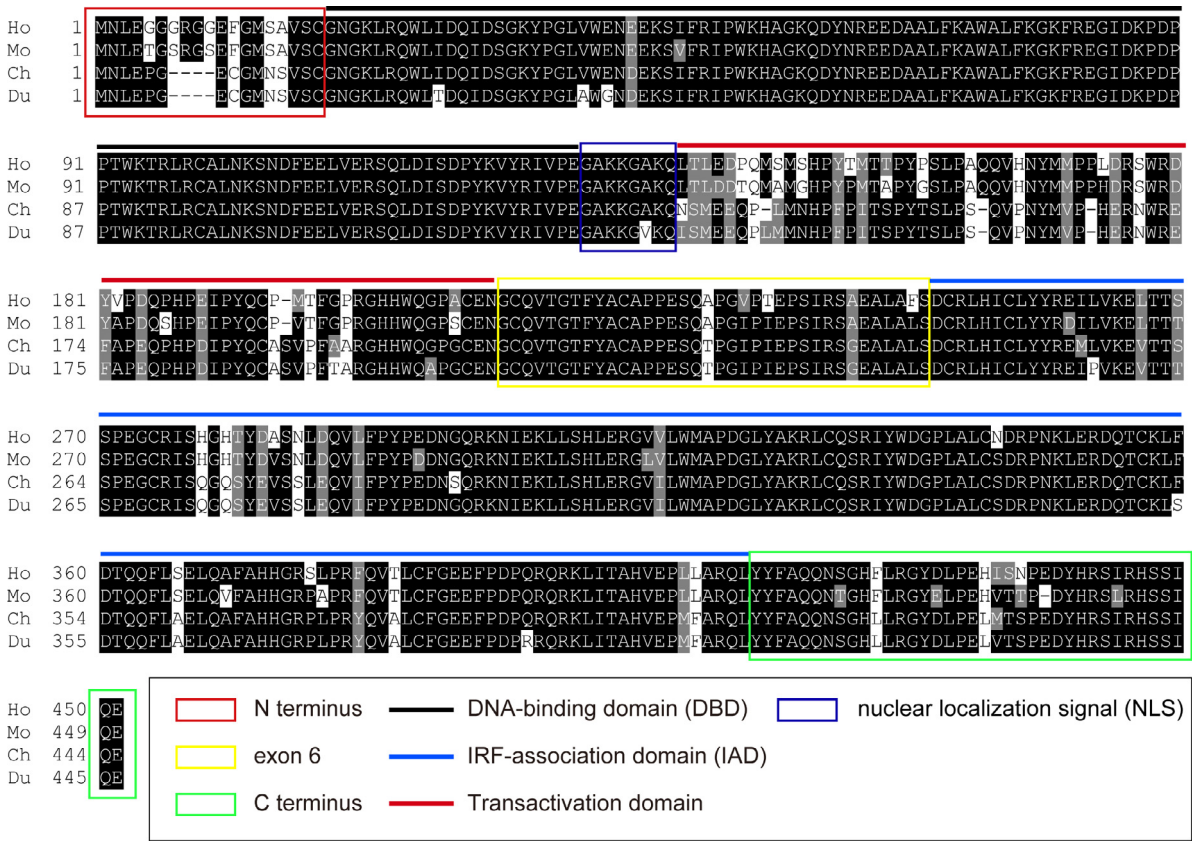


Figure 1. Alignment of the deduced AA sequence of duIRF4 with other animals. DuIRF4 can be divided into seven regions: N terminus, DNA-binding domain, putative nuclear localization signal, transactivation domain, exon 6, IRF-association domain and C terminus. Figure 1 annotates the three conserved domains of the IRF4 protein sequence in different ways: the N-terminal DBD domain (the black line in Figure 1), the C-terminal IAD domain (the blue line in Figure 1) and the NLS domain (blue box in Figure 1). Abbreviations: Ch, Gallus gallus; Du, Cherry Valley Duck; Ho, Homo sapiens; Mu, Mus musculus.

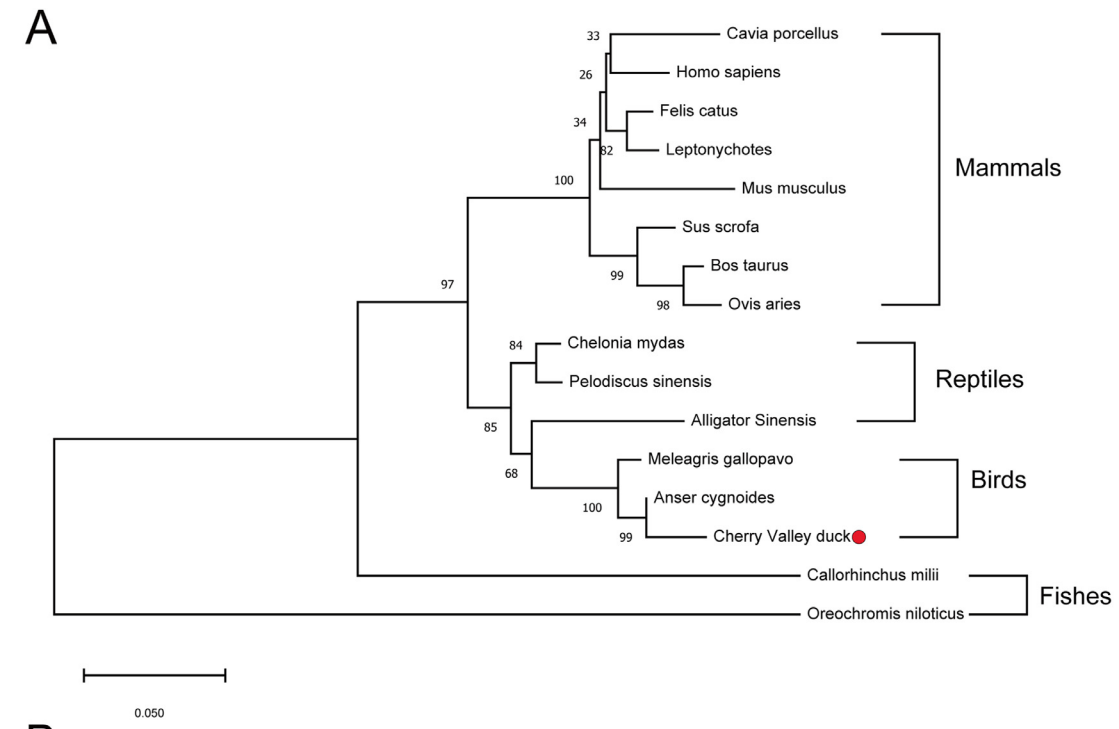
different tissues was detected by qRT-PCR. It can be seen from the results that duIRF4 can be widely expressed in tissues, with the highest expression in the spleen, followed by the bursa of Fabricius, and lower expression in the skin and brain (Figure 3).

Expression Profiles of duIRF4 in the Viral Infected Ducks

To examine the role of IRF4 in the antiviral response, the expression changes of IRF4 in the spleen and brain were detected after viral infection. The results showed that the IRF4 in the spleen and the brain was rapidly upregulated at 1, 3, 5 dpi with DTMUV and DPV, and the expression level of IRF4 upregulation in the brain was greater than that in the spleen. After inoculation with NDRV, IRF4 in the spleen showed a downward trend at 1 dpi, and the downward regulation reached a very significant level. The spleen rapidly increased at 3, 5 dpi, and the brain also rapidly increased 1, 3, 5 dpi. The expression of IRF4 in the spleen and brain of Cherry Valley ducks reached extremely significant differences at 1, 3, 5 dpi with DTMUV ($P < 0.01$), and the upregulation reached the maximum on the third day in the spleen (96.5-fold, $P < 0.01$; Figure 4A); the maximum upregulation (1,572.4-fold, $P < 0.01$; Figure 4B) in the brain at 5 dpi. After being infected by NDRV, the content of duIRF4 in the spleen gradually increased at 1, 3, 5 dpi, but the overall content was low (Figure 4C). DuIRF4 levels in the brain increased significantly and reached the peak value at 1 dpi, and then decreased gradually (Figure 4D). After being infected by DPV, the upregulation level of duIRF4 in the spleen was low, below 10-fold (Figure 4E), but it continued to increase during the 3 d of testing. However, the IRF4 in the brain was generally upregulated after infection, but the up-regulation level gradually decreased at 1, 3, 5 dpi, and the highest level at 1 dpi (Figure 4F). These results all indicate that IRF4 is involved in the body's antiviral immunity.

DISCUSSION

IRFs are a type of transcription factor that was originally discovered as a transcription factor for type I IFN. The mammalian IRFs protein family includes 9 members from IRF-1 to IRF-9. They participate in the function regulation of a variety of immune cells and play an important role in both physiological and pathological conditions (Negishi et al., 2018). With the discovery of the signal transduction pattern recognition receptor (**PRR**) used by the innate immune system to recognize pathogen-associated molecular patterns (**PAMP**), IRF has received widespread attention as an important regulator of PRR activation. We found that the duIRF4



B

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1		91.0	86.3	75.4	85.6	87.4	84.6	86.7	90.8	84.0	60.6	86.3	87.2	91.9	91.0	89.2	**1**
2	9.6		86.3	75.2	85.8	87.6	85.6	87.4	98.4	84.8	62.0	86.5	87.2	93.3	93.0	98.2	**2**
3	15.2	15.2		75.2	91.8	94.5	94.0	93.8	86.5	90.6	59.7	98.0	96.7	88.1	88.5	84.5	**3**
4	29.9	30.1	30.2		74.9	76.5	76.0	76.3	76.1	74.7	58.0	74.3	75.6	78.2	78.2	74.1	**4**
5	16.1	15.8	8.7	30.5		94.2	93.7	93.8	86.5	90.8	61.2	91.3	92.2	88.3	88.5	84.0	**5**
6	13.8	13.5	5.8	28.2	6.0		96.1	98.2	87.8	92.3	60.8	93.8	96.5	90.6	90.8	85.8	**6**
7	17.3	16.1	6.3	29.0	6.6	4.0		96.1	85.5	92.5	62.4	93.0	94.0	88.5	88.8	83.6	**7**
8	14.7	13.8	6.5	28.5	6.5	1.8	4.0		87.8	92.3	61.8	93.1	95.8	90.6	91.0	85.6	**8**
9	9.9	1.6	14.9	28.9	15.0	13.3	16.1	13.3		84.5	62.6	86.5	87.8	93.5	93.3	96.6	**9**
10	18.0	17.0	10.1	30.9	9.8	8.2	7.9	8.2	17.4		62.6	89.8	90.8	87.0	87.3	83.3	**10**
11	55.3	52.6	57.2	60.8	54.1	54.9	51.8	53.1	51.4	51.5		59.4	60.6	61.8	61.8	60.8	**11**
12	15.2	14.9	2.0	31.6	9.3	6.5	7.4	7.2	14.9	11.0	57.7		95.3	87.6	88.1	84.7	**12**
13	14.1	14.1	3.4	29.5	8.2	3.6	6.3	4.3	13.3	9.8	55.3	4.8		90.3	90.6	85.4	**13**
14	8.6	7.1	13.0	25.8	12.8	10.1	12.5	10.1	6.8	14.3	53.1	13.5	10.4		98.2	91.5	**14**
15	9.6	7.3	12.5	25.8	12.5	9.9	12.2	9.6	7.1	14.0	53.1	13.0	10.1	1.8		91.3	**15**
16	11.7	1.8	17.4	31.8	18.0	15.7	18.5	16.0	3.5	18.9	54.9	17.1	16.3	9.1	9.3		**16**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	

Divergence

1 Alligator Sinensis
2 Anser cygnoides
3 Bos taurus
4 Callorhinchus mydas
5 Cavia porcellus
6 Felis catus
7 Homo sapiens
8 Leptonychotes
9 Meleagris gallopavo
10 Mus musculus
11 Oreochromis
12 Ovis aries
13 Sus scrofa
14 Chelonia mydas
15 Pelodiscus sinensis
16 Cherry Valley duck

Figure 2. Phylogenetic analysis and sequence similarity of IRF4. The duIRF4 is marked with a red dot in Figure 2, and the homology with other animal IRF4 sequences is shown by the phylogenetic tree. Download the coding region sequence of IRF4 of other animals from the NCBI website, use Editseq software to convert it into a text file, and use the neighbor-joining algorithm in MEGA5.1 to construct the phylogenetic tree. The accession numbers of the sequences used in Genbank are shown in Table 2. The animals involved include mammals, birds, reptiles, and fish.

contains 3 conserved functional domains: the N-terminal DBD domain, the C-terminal IAD domain, and the NLS domain. The DBD of IRFs has extensive homology. Studies have shown that the DBD domain of IRFs is similar to the DBD of Myb protein and can recognize and bind DNA by forming a helix-turn-helix domain (Hu et al., 2013). In addition, the C-terminal IAD domain is a variable region, which is essential for determining the interaction between IRFs and other transcription factors or cofactors, thereby giving each IRF protein specific transcriptional activity and biological function. The phylogenetic tree shows duIRF4 only exhibited 26% identities to *Homo sapiens*, 84% to *Chelonia mydas*, and 74.1% to *Callorhinchus milii*. Besides, duIRF4 exhibits the highest homology with *Meleagris gallopavo*. This fact suggests that duIRF4 has a close relationship with other birds. Since IRF4 plays a role in the signal pathway that recognizes PAMP in the innate immune system, our study of its distribution in tissues can provide a reference for studying its specific functions. In our research, we found that duIRF4 is present in all healthy tissues, with the highest expression in the spleen, followed by the bursa of Fabricius, which are both of immune organs. The spleen is the body's largest immune organ. The main function of the spleen is to participate in the immune response, swallow and remove senescent red blood cells, foreign bodies and bacteria, and produce lymphocytes and monocytes, thereby

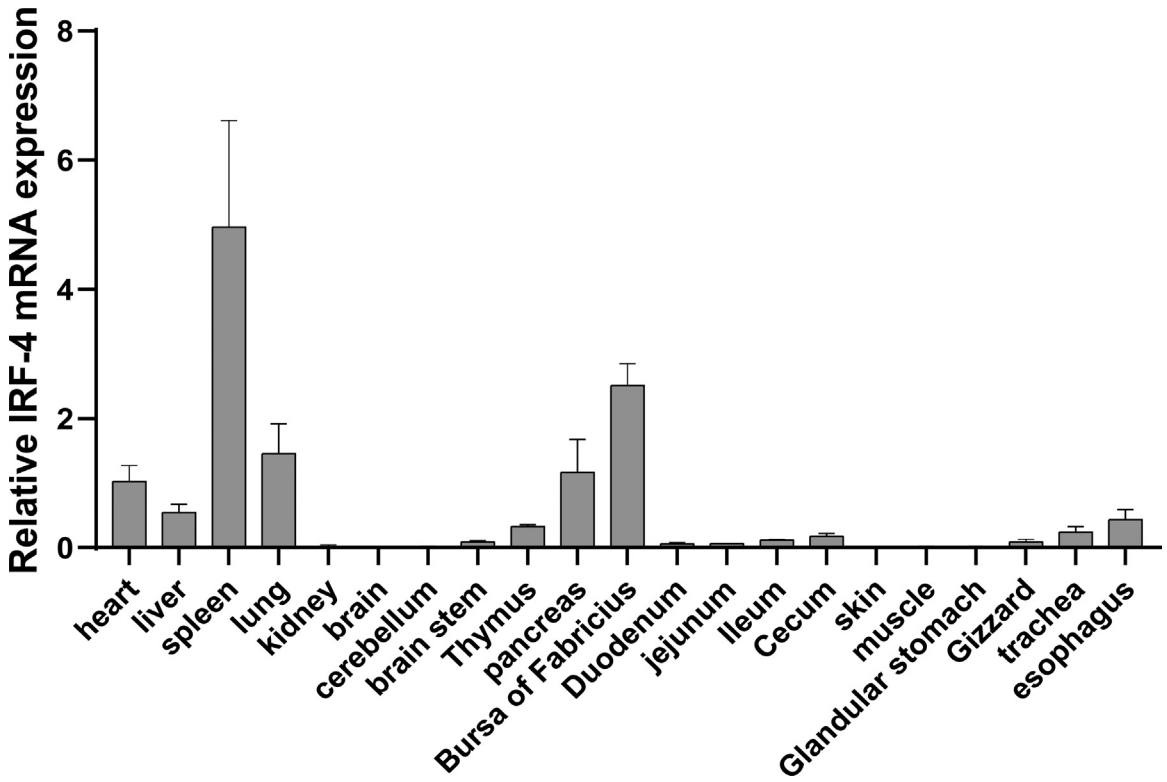


Figure 3. Tissue distribution of duIRF4 transcripts in healthy Cherry Valley duck. The expression of IRF4 mRNA in various tissues of healthy Cherry Valley ducks was detected by qRT-PCR. The obtained mean uses β-actin gene as internal reference and heart as reference tissue. The quantification of the relative expression level of the target gene and the reference gene β-actin is calculated by the $2^{-\Delta\Delta Ct}$ method. All samples are set to 3 replicates to ensure the accuracy of the experiment.

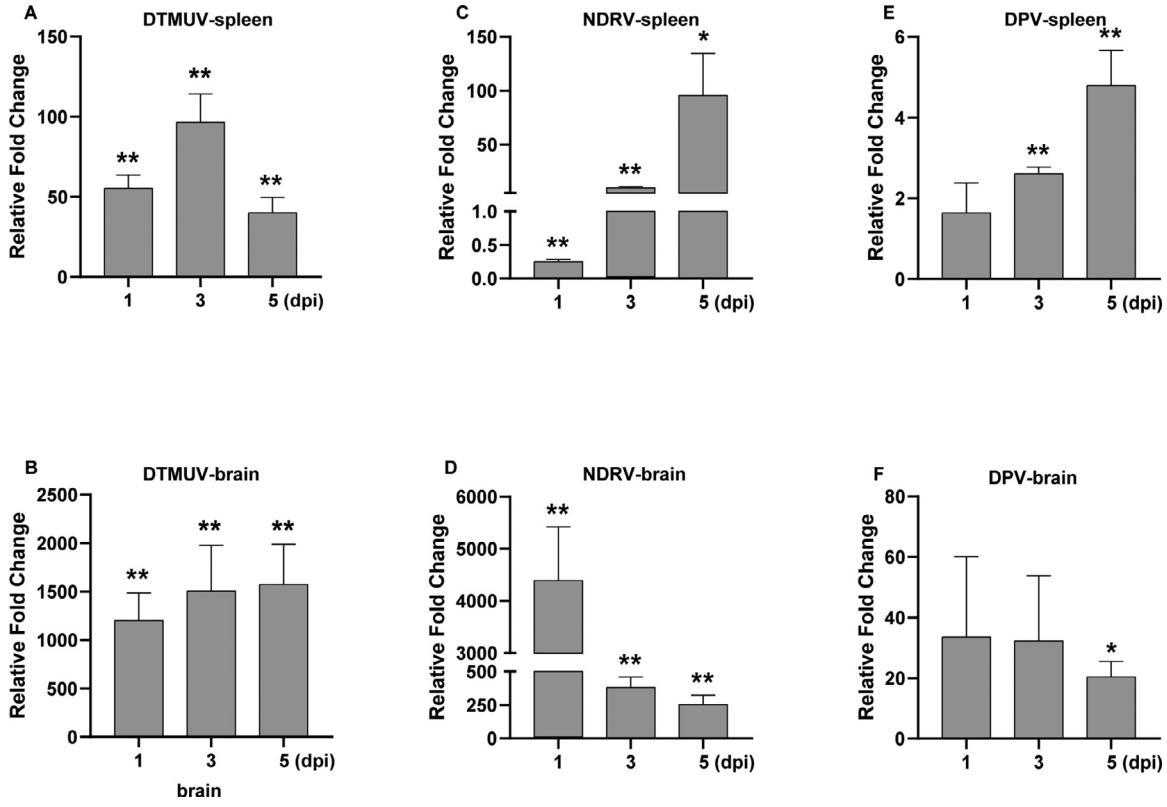


Figure 4. Analysis of duIRF4 transcript after infection with the three viruses (A) DTMUV-spleen, (B) DTMUV-brain, (C) NDRV-spleen, (D) NDRV-brain, (E) DPV-spleen, and (F) DPV-brain. The relative expression levels were calculated with the $2^{-\Delta\Delta Ct}$ method. The data of each group are expressed as mean±SD, and the students' t test is used to analyze the difference between the two groups of data. In the figure, "*" indicates that the statistical value of $P < 0.05$ is considered to be significant, and "**" indicates that the statistical value of $P < 0.01$ is considered to be extremely significant.

participating in the body's cellular and humoral immunity (Groom, 1987; Chadburn, 2000). However, the distribution of IRF4 in different animals and different stages of tissues will be different. The detailed biological function of duIRF4 tissue distribution needs further study. This study examined the changes in the content of duIRF4 in the spleen and brain of Cherry Valley ducks infected with DTMUV, DPV, and NDRV to clarify whether duIRF4 participates in the host's antiviral immune response. Because these 3 viruses all cause neural and digestive features, we chose the spleen and brain as targets to detect the changes in the content of duIRF4 in the spleen and brain and found that the content of duIRF4 in the spleen and brain has changed significantly after virus infection. The expression of duIRF4 in the spleen and brain reached extremely significant differences at 1 to 5 dpi with DTMUV. Relatively speaking, the upregulation in the brain is more significant. However, duIRF4 in spleen reached maximum expression levels at 3 dpi and in brain reached maximum expression levels at 5 dpi. This indicates that duIRF4 in the spleen and brain is involved in anti-DTMUV infection, but duIRF4 in the spleen is involved in the antiviral immune response in the early phases of DTMUV infection. After being infected by DPV, although it is upregulated in the spleen and brain, the upregulation level is much lower than the upregulation level after DTMUV infection. In addition, the upregulated level of duIRF4 in the spleen gradually increased over time, while the upregulation level in the brain gradually decreases. This indicates that duIRF4 in the spleen is involved in the antiviral immune response in the early phases of DPV infection. Besides, this shows that duIRF4 in the spleen and brain is also involved in anti-DPV infection, but its role is far less powerful than its role in anti-DTMUV. After infection with NDRV, the level of duIRF4 in the spleen showed a downward trend at 1 dpi, and the downward adjustment reached a very significant level. Besides, the change trend of duIRF4 levels in the spleen and brain after NDRV infection is similar to that of DPV infection, but the degree of upregulation is much greater than that after DPV infection. All these results indicate that duIRF4 participates in the antiviral immune response to the above 3 viruses. This indicates that IRF4 participates in the host's innate immune response in Cherry Valley ducks, which means that IRF4 may play a pivotal role in the immune system.

Through the preliminary study on the function of IRF4 in Cherry Valley ducks, it can be determined that duIRF4 plays an important role in the body's antiviral process. However, the signal pathway mediated by IRF4 of Cherry Valley duck, the mechanism of action in the antiviral process, and the effect on upstream and downstream cytokines needs further research to determine.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31972664), the China Postdoctoral Science Foundation (2018M632268 and 2019T120404) and the Local Science and Technology Development Fund Project Guided by the Central Government of Shandong Province (YDZX20203700004857).

Author contributions: Xinyu Zhai and Tianqi Hong wrote the manuscript and performed the most of the experiments. Tingting Zhang and Tianxu Li provided help for the experiment and writing, Jinchao Wang and Bin Xing helped complete the animal experiment, Xiuyuan Wang and Runchun Miao collected samples. Liangmeng Wei designed the study and polished the article.

Ethics statement: This animal study was reviewed and approved by Shandong Agricultural University Animal Care and Use Committee (no. SDAUA-2017-045).

DISCLOSURES

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

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