Evolutional conservation of molecular structure and antiviral function of a type I interferon, IFN-kappa, in poultry

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A B S T R A C T

IFN-kappa (IFN-κ) is a type I IFN expressed by keratinocytes, monocytes and dendritic cells with important roles during the innate immune response period. This research was conducted to elaborate the evolution and characteristics of IFN-κ in poultry. Chicken IFN-κ is located on the sex-determining Z chromosome, which is greatly different from mammals. Poultry IFN-κ cluster together in a species-specific manner through positive selection pressure and share only 19–33% homology with mammalian IFN-κ and poultry other type I IFN. Both chicken and duck IFN-κ was constitutively expressed in spleen, skin, lung, and peripheral blood mononuclear cells (PBMC), as well as being significantly induced after treatment with virus in PBMC. Biologically, poultry IFN-κ has antiviral activity against VSV in chicken embryonic fibroblasts and duck embryonic fibroblasts (CEF and DEF) cells, and induces the expression of IFN stimulated genes (ISGs). After treatment with JAK1 inhibitor, the ISGs expression can be down-regulated. Overall, our research on poultry IFN-κ not only enriches the knowledge about IFN-κ but also facilitates further research on the role of type I IFNs in antiviral defense responses in poultry.

1. Introduction

Interferons (IFNs) are expressed by many cell types and play a key role in the immune responses against viral infections, and are classified as type I, type II and type III IFN according to their function and binding receptors. Type I IFNs include several subclasses: IFN-α, IFN-β, IFN-ω, IFN-τ, IFN-δ, IFN-ε, IFN-κ, IFN-limitin. In humans, there are at least 13 structural IFN-α genes encoding functional proteins and single IFN-β, IFN-ω, IFN-ε, and IFN-κ genes (Kuruganti et al., 2014; Walker and Roberts, 2009; Woelk et al., 2007). Similarly, in mice, there are 14 IFN-α genes and single IFN-β, IFN-ε, and IFN-κ genes (Walker and Roberts, 2009), which lack an IFN-ω gene. In cows, IFN-β and IFN-ω also possess multiple genes, lacking the IFN-limitin gene but with an extra IFN-τ gene compared with humans and mice (Walker and Roberts, 2009). In chickens, there are only 12 IFN-α genes and single IFN-β and IFN-κ genes (Goossens et al., 2013; Santhakumar et al., 2017). As described above, the mammals IFN system evolved with the species, which is conserved among them but still has some differences, while the poultry

IFN system has great evolutionary differences with that of mammals in the IFN locus arrangement and gene structure.

IFN-κ is expressed by keratinocytes, monocytes and dendritic cells (DCs), displays a tight tropism for keratinocytes and specific lymphoid populations and shares some biological functions with other type I IFNs, including the antiviral effect and regulation of cytokine expression (LaFleur et al., 2001; Nardelli et al., 2002), which is ~30% identical to the other type I interferon family members (DeCarlo et al., 2010; LaFleur et al., 2001), with relatively weak antiviral activity, and specific expression in skin and mucosa. A pooled genome-wide scan implicated the IFN-κ locus in systemic lupus erythematosus susceptibility, and IFN-κ variants could influence type I IFN producing plasmacytoid dendritic cells in affected skin (Harley et al., 2010). IFN-κ is down-regulated in cervical keratinocytes harboring human papillomavirus (HPV) that target IFN-κ by different pathways in keratinocytes to inhibit both antiviral ISGs and pathogen recognition receptors (DeCarlo et al., 2010; Reiser et al., 2011; Rincon-Orozco et al., 2009), and IFN-κ was not detectable in healthy skin but was strongly expressed in allergic

contact dermatitis and lichen planus-affected skin (Scarponi et al., 2006), which suggest IFN-κ presence or deficiency has a relationship with disease progression and can be used as a disease indicator.

Studies on IFN-κ are mostly focused on mammals, and the human IFN-κ related to HPV infection is most studied. Interestingly, IFN-κ is also distributed in poultry with limited type I IFN existing in poultry. Genetic, evolutionary and syntenic analyses indicate that chicken IFN-κ has conserved genetic features and promoter binding sites, which can activate the ISRE promoter and induce the transcriptional activation of ISGs (IFN stimulated genes) possibly through the activation of STAT1 (Santhakumar et al., 2017), but while poultry IFN-κ shares similar characteristics, it has not been elaborated clearly. In this study, we investigated the type I IFN locus, molecular characterization, and antiviral function of poultry IFN-κ, which was demonstrated to evolve from the same branch and to be located on the sex-determining Z chromosome, exerting antiviral activity through the JAK-STAT pathway. These findings provide the evidence for the evolutionary and antiviral function of poultry IFN-κ.

2. Materials and methods

2.1. Viruses, cells, animals and protein

CEF (Chicken Embryonic Fibroblasts) and DEF (Duck Embryonic Fibroblasts) cells were prepared with chicken and duck embryos purchased from Harbin Veterinary Research Institute (Heilongjiang, China). DF1 cells (UMNSAH/DF1 Chicken Fibroblast Cell Line) were preserved in our laboratory. VSV (Vesicular Stomatitis Virus) was purchased from the China Institute of Veterinary Drug Control. NDV (Newcastle Disease Virus) Lasota vaccine strain was purchased from the Wei Ke Co. Ltd. (Heilongjiang, China). Chickens (Binbai 428 layers, Female, 20 weeks old) were acquired from Hua Xing Poultry Co. Ltd. (Heilongjiang, China). Ducks (Pekin ducks, Male, 10 weeks old) were purchased from Harbin Veterinary Research Institute. The animals used in this study were approved by the Laboratory Animal Ethical Committee of Northeast Agricultural University. The animals were treated in accordance with the Chinese Regulations of Laboratory Animals and the Guidelines for the Care of Laboratory Animals. BoIFN-αA was expressed and preserved in our laboratory, and confirmed no antiviral activity against VSV on CEF and DEF cells.

2.2. Location of poultry IFN-κ gene

The human IFN-κ amino acids sequence (GenBank: NP_064509) was used as the query sequence, and the Blast Genomes in the Blastn suite with parameter settings as BLAST algorithm was performed. The annotations of type I IFN gene fragments from the poultry genome sequence database (tax id:9031 (chicken), tax id:8839 (duck), tax id:8845 (goose)) were identified to be similar to other animals IFN gene sequences published online (Altschul et al., 1997), and the poultry type I IFN locus was organized and constructed. Finally, the IFN-κ genes selected from the poultry type I IFN locus were identified and analyzed.

2.3. Clone and sequence analysis

After locating the poultry IFN-κ genes in the genome sequence database, two pairs of specific primers (Table 1) were designed to amplify the ChIFN-κ, DuIFN-κ, GoIFN-κ gene. Genomic DNA extracted from the liver of poultry were separately used as templates in PCR amplification with the following thermal profile: initial denaturation at 94 °C for 5 min, 30 amplification cycles of denaturing at 98 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, followed by an elongation step at 72 °C for 10 min. Then the PCR products cloned into the pEASY Blunt-T Vector (TransGen, Beijing, China) were separately identified and sequenced. The sequence characteristics were analyzed with bioinformatics software. Open reading frame (ORF) was searched

Table 1
Nucleotide Sequence of the PCR primers used in this study.

Primer	Sequence (5′-3′)	Size
ChIFNK-F	ACAGTGTTATCCCACTGCTAAG	
ChIFNK-R	TGCCCCAAGACAGATCATAGTAT	1017 bp
DGIFNK-F	AGCAGCTTATCTTTACCATG	
DGIFNK-R	GTACCTTAGTTGTAAAGCTTCC	583 bp
ChIFNK-ORFBF	ATCGGATCC$_{BamH\ I}$CTTACCTGTAATCACCTTCC	
ChIFNK-ORFXR	ACACTCGAG$_{Xho\ I}$TTACTTGTAAAGCTTCCTTATG	510 bp
DGIFNK-ORFBF	GTGGGATCC$_{BamH\ I}$CTTAACTGTAATCACCTTCC	
GoIFNK-ORFNF	ACCGTGCATATG$_{Nde\ I}$CTTAACTGTAATCACCTTCC	
DGIFNK-ORFXR	CTCCTCGAG$_{Xho\ I}$TTAGTTGTAAAGCTTCCTCACG	519 bp

Note: The restriction enzyme sites that were introduced in primers are underlined.

by the ORF Finder algorithm (http://www.ncbi.nlm.nih.gov/gorf/). Editseq and Megalign programs of the Lasergene 11 package (DNAStar, Inc., USA) and ClustalX were used to sequence alignment and for analysis. Signal peptide (http://www.cbs.dtu.dk/services/SignalP/), glycosylation sites (http://www.cbs.dtu.dk/services/NetNGlyc/and http://www.cbs.dtu.dk/services/NetOGlyc/) and 3D structure (http://swissmodel.expasy.org/workspace/) were predicted online.

2.4. Molecular evolutionary analysis

IFN-κ gene coding sequences available in the NCBI (http://www.ncbi.nlm.nih.gov) and Ensembl databases were collected and aligned altogether with the poultry IFN-κ sequences obtained in this study by using the program ClustalW. Then phylogenetic trees were constructed with MEGA 7.0 using the UPGMA method (Kumar et al., 2016). Evolutionary analyses were conducted in MEGA7.0 using the Maximum Likelihood method based on the Tamura 3-parameter model, and the availability of branch stem was verified with the Bootstrap by the parameter 500 with the best model selected first (Kumar et al., 2016).

Adaptive evolution analysis of IFN-κ was conducted in the Datamonkey Web server online (http://datamonkey.org/), and the software GARD (Kosakovsky et al., 2006a; b) implemented in the Datamonkey Web server was applied to look for any evidence of recombination in the IFN-κ alignment. IFN-κ in different species was analyzed pairwise using a dN/dS calculation tool (http://services.cbu.uib.no/tools/kaks) for calculation of synonymous (dS) or non-synonymous (dN) changes in the coding regions. Significant estimation (p < 0.05) of positive and purifying selections were also calculated using MEGA 7.0 (Tamura et al., 2011).

The gene synteny of bird, reptile, and mammal IFN-κ locus was analyzed using Genomicus (v90.01) (http://www.genomicus.biologie.ens.fr/genomicus-90.01/cgi-bin/search.pl) and duck IFN-κ was set as a reference to compare the conserved synteny between different species.

2.5. Transcriptional expression of poultry IFN-κ

Real-time PCR was conducted to analyze the tissue distribution of chicken and duck IFN-κ genes in their tissues. First, RNAs were isolated from the heart, liver, spleen, lung, kidney, thymus, small intestine, colon, ovary, testicle, skin, muscle, and brain of chicken and duck. Then the RNAs were reverse-transcribed with Prime-Script ™ RT reagent Kit with gDNA Eraser (Takara, Dalian, China) and subjected to real-time PCR as described previously (Gao et al., 2018) with specific primers spanning intron (ChNKIN and DuNKIN, Supplementary Table S1). The real-time PCR reactions were carried as follows: 50 °C for 2 min and 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. The gene expression levels were analyzed by the $2^{-\Delta\Delta CT}$ method. GAPDH was used as the internal reference gene, and the PCR product was extracted and sequenced.

Chicken and duck peripheral blood lymphocyte cells (PBMC) were