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Chicken interferon regulatory factor 1 (IRF1) involved in antiviral innate immunity via regulating IFN-β production

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ABSTRACT

Interferon regulatory factors (IRFs) is an important family for IFN expression regulating while viral infection. IRF1, IRF3, and IRF7 are the primary regulators that trigger type I IFN response in mammals. However, IRF3, which has been identified as the most critical regulator in mammals, is absent in chickens, and it is unknown whether IRF1 is involved in type I IFN signaling pathways in IRF3-deficient chicken cells. Here, we identified chicken IRF1 (chIRF1) as a critical IFN-β mediator in response to viral infection. Overexpression of chIRF1 activated IFN-β intensively and suppressed AIV and NDV viral replication. Moreover, the mRNA levels of IFN-β and ISGs increased during chIRF1 overexpression. In addition, deletion mutant analysis revealed that the first four domains of chIRF1 are indispensable for IFN-β induction. Together, our studies demonstrate that chIRF1 is an important regulator of IFN-β and is involved in chicken antiviral innate immunity.

1. Introduction

When pathogens invade a host, innate and/or adaptive immunity are activated to protect the body from infection. Innate immunity provides a critical effect to respond to the invading pathogen by producing interferons (IFNs) rapidly (Zhou et al., 2014). Pattern recognition receptors (PRRs) from the host cell recognize the pathogen-associated molecular patterns (PAMPs) of the invading viruses and then evoke various adaptor-dependent signaling cascades. As a class of PRRs, retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), such as RIG-I and melanoma differentiation-associated gene 5 (MDA5), recognize the dsRNA component in cell cytoplasm, which triggers mitochondrial antiviral signaling (MAVS)-dependent IFN signaling. Toll-like receptors (TLRs), located in cell surface or endosomal, detect RNA or some special DNA like unmethylated CpG DNA, mediate IFN pathways depending on TIR-domain-containing adaptor inducing interferon-β (TRIF) or myeloid differentiation factor 88 (MyD88). These signaling cascades then center on TBK1 and IKKα/β to promote the NF-κB and the IFN regulatory factor (IRF) family members, especially IRF3 and/or IRF7, leading the type I IFNs expression (Gürtler and Bowie, 2013).

The IRF gene family encodes diverse transcription factors and has multiple biological functions. Till now, 11 IRF family members have been reported in fish. In mammals, 10 members have been identified, and two members (IRF10 and IRF11) are missing (Stein et al., 2007). By contrast, only 8 members have been found in chickens, and IRF3 and IRF9 are absent in chickens and other avian species (Huang et al., 2010; Cormican et al., 2009). These factors mainly regulate the expression of IFN and IFN-stimulated genes (ISGs) by binding to specific IFN-stimulated regulatory elements (ISREs) (Mamane et al., 1999; Taniguchi et al., 2001; Nair et al., 2017). In humans and mice, IRF3 and IRF7 have been identified as the most important transcription factors that regulate type I IFN expression via phosphorylation and heterodimerization, then translocate into the nucleus for viral invading (Kim and Zhou, 2015). Although IRF3 is deficient in chickens, a previous study (Liu et al., 2015) showed that IRF7 is involved in response to low pathogenic AIV infection in DF-1. As the first member identified in the IRF family, IRF1 can activate the IFN-β expression and is involved in hematopoietic differentiation, antiviral and antibacterial responses, and cytokine signaling (Nguyen et al., 1997; Li et al., 2015). IRF1 has been reported to regulate IFN antiviral response via the MyD88 signaling pathway in zebrafish (Feng et al., 2015). Moreover, *Irf1*$^{-/-}$ mice exhibited greater chikungunya virus and Ross River virus infection in muscle tissues compared to normal mice (Nair et al., 2017), and in ducks, IRF1 restricts viral replication effectively via inducting IFN and upregulating antiviral ISGs (Qian et al., 2018). This study illustrates that IRF1 is involved in regulating IFN-β production during viral infections in IRF3-null chicken cells.

In this study, we cloned the chIRF1 gene and first revealed the

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protein's functional domains to activate IFN-β in chickens. Moreover, chIRF1 may depend on the MDA5 pathway to reduce AIV and NDV replication by regulating IFN-β production. Because the exact mechanism by which chIRF1 induces IFN-β is still poorly understood, this study investigated the chIRF1 effects on induction of IFN-β and its antiviral activity. Further research is needed to identify the interaction molecules of chIRF1 in the IFN pathway and the relationship between IRF1 and IRF7 in IRF3-null chickens. This study provides key insights into the role of chIRF1 plays in IFN immune response.

2. Materials and methods

2.1. Cells and virus

DF-1(ATCC), a chicken embryonic fibroblast cell line. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone, China) containing with 10% Fetal Bovine Serum (FBS) (Gibco, USA), 1% Penicillin-Streptomycin (Gibco, USA). The cells incubator was maintained at 37 °C with 5% CO_2. A virulent strain of NDV, Herts/33, was the gifts of Professor Ding (Shanghai Veterinary Research Institute, China). The A/Chicken/Shanghai/010/2008 (H9N2) virus (SH010) was isolated from chickens in our lab. Viruses were purified, propagated, and stored as described in our preceding study (Cheng et al., 2015a).

2.2. Cloning and bioinformatics analysis of chIRF1

According to the chIRF1 sequence (GenBank NO. NM_205415.1), primers chIRF1 ORF -23U and chIRF1 ORF 952L described in Table 1, were designed and used for amplifying chIRF1 cDNA fragments by real-time PCR (RT-PCR) from total chicken spleen RNA. The PCR fragment was ligated into a pTOPO-Blunt vector for sequencing. The amino acid sequences were aligned using BioEdit and MegAlign software.

2.3. Construction of plasmids

For luciferase assays, the pGL-IFN-β-Luc report plasmid was the firefly luciferase reporter vector pGL3.0 (Promega) that containing the chicken IFN-β promoter fragment (−158 to +14) between *NheI* and *BglII* sites. Renilla plasmid pRL-TK (Promega) was the internal control. To get the mutations that lacking different domains of chIRF1, various primers (Table 1) were designed based on the full-length chIRF1 sequence. The expression plasmids pcDNA-chIRF1-Myc and the mutation forms IRF1-1-60, IRF1-1-120, IRF1-1-141, IRF1-1-253, IRF1-1-293, IRF1-1-303, IRF1-11-313, IRF1-61-313, and IRF1-141-313 were created by insertion full-length chIRF1 or it's corresponding truncated DNA fragments into a pcDNA3.0 vector, which containing a Myc flag.

2.4. Quantitative real-time PCR analysis

RNA was extracted using the MiniBEST Universal RNA Extraction Kit (Takara). Total RNA was reverse transcribed into cDNA using PrimeScript™ RT Master Mix (Takara). The quantitative real-time PCR (qRT-PCR) was performed on Applied Biosystems 7500 Real-Time PCR system, and the reaction mixture was composed of 10 μl 2 × SYBR Green PCR master mix (Takara), 9.4 μl nuclease-free water, 1 μl cDNA, 0.1 μl each gene-specific primer (100 mM; Table 1), and 0.4 μl Dye II. Threshold cycle (Ct) values were normalized using β-actin as an internal reference with the comparative Ct ($2^{-\Delta\Delta Ct}$) method.

2.5. Luciferase reporter assays and western blotting

DF-1 cells were plated in 24-well plates and incubated until 90%–95% confluent; then they were co-transfected with 0.2 μg indicated plasmids or empty vector, together with pGL-IFN-β-Luc (0.15 μg/well) and pRL-TK (0.07 μg/well), by Lipo2000 (Invitrogen). Cells were lysed 24 h post transfection, the luciferase activity was carried out

Table 1
Primers used in this study.

Primers	Purpose	Sequence (5′-3′)
chIRF1 ORF -23U	To obtain sequence	ACAGGATCTCACGGCGAGGAAACATG
chIRF1 ORF 952L		AAGCAAGGGTTTACAAGCTGCAGGAG
EcoR I chIRF1 U	Cloning	TAGTAGTGTGGTGGAATTCATGCCCGTCTCAGGATGC
Xho I chIRF1 L		GAAGGGCCCTCTAGACTCGAGCAAGCTGCAGGAGATGGCCT
chIRF1 11–313 U	Construct truncated forms of chIRF1	TAGTCCAGTGTGGTGGAATTCATGTGGTTGGAAATG
chIRF1 61–313 U		TAGTCCAGTGTGGTGGAATTCATGCATACAGGAAGA
chIRF1 141–313 U		TAGTCCAGTGTGGTGGAATTCATGGATATGAGGATG
chIRF1 1–60 L		GAAGGGCCCTCTAGACTCGAGGATGGCCCAGCTCCG
chIRF1 1–120 L		GAAGGGCCCTCTAGACTCGAGCTTCTGGTCCTTTGT
chIRF1 1–141 L		GAAGGGCCCTCTAGACTCGAGCTCATACAACTTTCT
chIRF1 1–253 L		GAAGGGCCCTCTAGACTCGAGGCCTTTCCCCTCAAC
chIRF1 1–293 L		GAAGGGCCCTCTAGACTCGAGGCTCTTCTGGTCCAT
chIRF1 1–303 L		GAAGGGCCCTCTAGACTCGAGGACGGTGTCCAGCCA
qchIRF1 U	qRT-PCR	ACGGCGGACTCACCGCTCCCCTC
qchIRF1 L		TCAGTCAAGTTCAAATGGCATTTG
qIFN-β U		CCTCAACCAGATCCAGCATT
qIFN-β L		GGATGAGGCTGTGAGAGGAG
qchIRF7 U		GCCTGAAGAAGTGCAAGGTC
qchIRF7 L		CTCTGTGCAAAACACCCTGA
qchIL-1β U		CAGCACCTCAGCGAAGAG
qchIL-1β L		CTGTGGTGTGCTCAGAATCCA
qchIL-8 U		ATTCAAGATGTGAAGCTGAC
qchIL-8 L		AGGATCTGCAATTAACATGAGG
qchMyD88 U		AGCGTGGAGGAGGACTGCAAGAAG
qchMyD88 L		CCGATCAAACACACACAGCTTCAG
qchMAVS U		CACCCACGAGGTCCATGTG
qchMAVS L		TGCTTCATCTGGGACATCATTG
qchMx-1 U		GTTTCGGACATGGGGAGTAA
qchMx-I L		GCATACGATTTCTTCAACTTTGG
qchMDA5 U		TGAAAGCCTTGCAGATGACTTA
qchMDA5 L		GCTGTTTCAAATCCTCCGTTAC
qβ-actin U		CAGACATCAGGGTGTGATGG
qβ-actin L		TCAGGGGCTACTCTCAGCTC