Molecular characterization and immune functional analysis of IRF2 in common carp (Cyprinus carpio L.): different regulatory role in the IFN and NF-κB signalling pathway

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Abstract

Background: Interferon regulatory factor 2 (IRF2) is an important transcription factor, which can regulate the IFN response and plays a role in antiviral innate immunity in teleost.

Results: In the present study, the full-length cDNA sequence of IRF2 (CcIRF2) was characterized in common carp (Cyprinus carpio L.), which encoded a protein containing a conserved DNA-binding domain (DBD) and an IRF-associated domain (IAD). Phylogenetic analysis showed that CcIRF2 was most closely related with IRF2 of Ctenopharyngodon idella. CcIRF2 transcripts were detectable in all examined tissues, with higher expression in the gills, spleen and brain. CcIRF2 expression was upregulated in immune-related tissues of common carp upon polyinosinic:polycytidylic acid (poly (I:C)) and Aeromonas hydrophila stimulation and induced by poly (I:C), lipopolysaccharide (LPS), peptidoglycan (PGN) and flagellin in the peripheral blood leucocytes (PBLs) and head kidney leukocytes (HKls). In addition, overexpression of CcIRF2 decreased the expression of IFN and IFN-stimulated genes (ISGs), and a dual-luciferase reporter assay revealed that CcIRF2 could increase the activation of NF-κB.

Conclusions: These results indicate that CcIRF2 participates in antiviral and antibacterial immune response and negatively regulates the IFN response, which provide a new insight into the regulation of IFN system in common carp, and are helpful for the prevention and control of infectious diseases in carp farming.

Keywords: Common carp (Cyprinus carpio L.), Interferon regulatory factor 2 (IRF2), Poly (I:C), Aeromonas hydrophila, IFN, NF-κB
2 (IRF2) has been characterized in rainbow trout (*Oncorhynchus mykiss*) [6], mandarin fish (*Siniperca chuatsi*) [7], snakehead (*Channa argus*) [8], zebrafish (*Danio rerio*) [9], stickleback (*Gasterosteus aculeatus*) [9], orange-spotted grouper (*Epinephelus coioides*) [10], Atlantic salmon (*Salmo salar*) [11], paddlefish (*Polyodon spathula*) [12], miyuki croaker (*Micthys miyui*) [13], grass carp (*Ctenopharyngodon idella*) [14], blunt snout bream (*Megalobrama amblycephala*) [15], Atlantic cod (*Gadus morhua*) [16] and golden pompano (*Trachinotus ovatus*) [5]. The expression of IRF2 can be upregulated by viral [5–8, 10, 12–14, 16] or bacterial [5, 10, 15] stimulation in different fish tissues, suggesting that IRF2 plays a role in host antiviral and antibacterial responses.

The IFN response is crucial to the antiviral innate immunity of teleost, which can be regulated by IRF2 in different ways. In zebrafish, the expression level of IFNa is increased by the knockdown of IRF2, which has been shown to negatively regulate IFNa signalling [17]. In Atlantic salmon, IRF2 acts as a negative regulatory factor for IFNa1 by competing with IRF-1 [11]. In grass carp, IRF2 can also bind the promoter of IFN via its DBD and downregulate the transcriptional activity of IFN [14]. However, IRF2 plays a positive role in regulating IFNa3 and IFNy expression in golden pompano [5, 18]. Interestingly, in large yellow croaker, IRF2 was found to induce IFNð and IFNh promoter activity but inhibit IFNc promoter activity [19].

Common carp (*Cyprinus carpio* L.) is a pivotal aquaculture fish species widely cultured in Asia and Europe. Several IRFs have been identified from common carp, including IRF1, IRF3, IRF5, IRF7, IRF9 and IRF10 [20–24]. *Aeromonas hydrophila* is a well-known fish pathogenic bacterium that can cause infection in a number of fish species [25], including common carp (*Cyprinus carpio*) [26], goldfish (*Carassius auratus auratus*) [27], yellow catfish (*Pelteobagrus fulvidraco*) [28] and channel catfish (*Ictalurus punctatus*) [29]. Furthermore, fish are becoming more and more susceptible to *A. hydrophila* because of the increasing intensive rearing methods used in the aquaculture [30]. The previous studies have reported that carp IRFs participated in the immune response against *A. hydrophila* [23]. Thus, in the present study, we identified the full-length cDNA sequence of IRF2 (*CcIRF2*) in common carp, and investigated its responsiveness to viral and bacterial immune stimulation both in vivo and in vitro. Meanwhile, we determined the roles of *CcIRF2* in regulating the IFN response and NF-κB signalling pathway. The present study will contribute to understand the innate immune system of fish, and is helpful for the prevention and control of infectious diseases in carp farming.

**Methods**

**Fish feeding and experimental challenge**

Common carp (about 200 g per fish) used in the study were purchased from a local fish farm in Jinan, China. After maintained in a fish feeding system (Qingdao Aiwen) at 20 °C for at least one week, 50 fish were anaesthetized by immersion in a 100 mg/l solution of MS222 (Sigma), and then evenly divided into two groups to injected intraperitoneally with 500 μl of 2.6 mg/ml poly (I:C) solution (Sigma) or 4.0 × 10⁵/ml *Aeromonas hydrophila*, which were inactivated with 0.5% formalin and suspended in PBS [23, 31, 32]. At 0, 3, 6, 12, 24, 48 and 72 h post-injection (hpi), three fish were anaesthetized and the liver, spleen, head kidney, skin, foregut and hindgut were collected.

**Cloning and analysis of the *CcIRF2* cDNA**

Total RNA was extracted from head kidney of common carp using TRIzol reagent (TIANGEN), and cDNA was synthesized using a FastQuant RT Kit (TIANGEN). A partial sequence of *CcIRF2* was amplified by PCR using the primers IRF2-F/IRF2-R (Table 1), which were designed based on the cDNA sequences of several known fish IRF2. Then, the full-length cDNA sequence was obtained by 3′- and 5′-Full RACE (rapid amplification of cDNA ends) method (TaKaRa). The domains of the *CcIRF2* protein were analysed using Simple Modular Architecture Research Tool (SMART, http://smart.embl-heidelberg.de), and multiple sequence alignment and phylogenetic analysis was performed using MEGA 6.0.

**Preparation of PBLs and HKLs from common carp**

Peripheral blood leucocytes (PBLs) and head kidney leucocytes (HKLs) of common carp were prepared according to previous studies [23, 33]. In brief, peripheral blood and head kidney cell suspensions were loaded onto freshly prepared 34%/51% Percoll (Sigma) density gradients and separated via centrifugation at 650 g for 30 min. The cells were resuspended and cultivated at 25 °C in Leibovitz’s L-15 medium with 10% foetal bovine serum (FBS), 100 UI/ml penicillin and 100 μg/ml streptomycin.

**Overexpression of *CcIRF2* in EPC cells**

The open reading frame (ORF) of *CcIRF2* was amplified by PCR using Phusion High-Fidelity DNA polymerase (PrimeSTAR), and purified fragments were ligated into the pCDNA3.1-EGFP vector and transformed into *E. coli* Top10 cells. The overexpression vector was extracted from positive clone using an endotoxin-free plasmid isolation kit (TIANGEN) and verified by sequencing. Epi-thelioma papulosum cyprini (EPC) cells were cultivated at 25 °C in M199 medium (HyClone) with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco).
Cells were transfected with pcDNA3.1-EGFP-CcIRF2 using X-tremeGENE HP DNA Transfection Reagent (Roche) at 2 μl/well following the manufacturer’s instructions.

**Real-time PCR analysis**

Real-time PCR was performed with TransStart Tip Green qPCR SuperMix (TransGen) in a Rotor-Gene Q PCR instrument (Qiagen) [23]. The expression levels of all genes were calculated relative to those of the 40S ribosomal protein S11 or the β-actin gene using the 2^(-ΔΔCt) method. The primers used are listed in Table 1.

**Dual-luciferase reporter assays**

The effects of CcIRF2 on the activation of NF-κB were performed using Dual-luciferase reporter assays. The 293T cells were cultivated at 37 °C in DMEM medium (HyClone) with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco), and transfected with reporter gene plasmids, pGL-NF-κB-luc and pGL-Renilla-luc, and the pcDNA3.1-EGFP-CcIRF2 vector using Lipofectamine 2000 (Invitrogen). 48 h after transfection, the Dual-Glo® Luciferase Reagent (Promega) was used to measure firefly and Renilla luciferase activity according to the manufacturer’s instructions.

**Statistical analysis**

Differences significance analysis were performed using one-way analysis of variance (ANOVA) or T-test in GraphPad Prism 6. All the data were homogeneous and normal, and P < 0.05 was considered as significative.

**Results**

**Cloning and characterization of CcIRF2**

The full-length cDNA of CcIRF2 (GenBank accession No. MW559072) consists of 1916 bp, including an ORF of 1005 bp that translates into a 334-amino acid putative peptide. The 5' and 3'-untranslated region (UTR) of the cDNA is 118 bp and 793 bp, respectively, and an mRNA instability motif (1861AATAA1865) is contained in the 3'-UTR. The predicted CcIRF2 protein contains a DBD (M1-S115) and an IAD (E211-F265) domain, with six tryptophan residues (Trp11, 26, 38, 46, 58 and 76) and a nuclear localization signal (NLS) in the DBD.

Multiple sequence alignment revealed that IRF2 amino acids sequences were conserved in all vertebrates, and significant homology was found in DBD (Fig. 1). The phylogenetic tree including IRF2 sequences from all known species was constructed using the neighbour-joining method, which was divided into multiple branches, and CcIRF2 was most closely related with IRF2 of C. idella (Fig. 2).

### Table 1 Primers used in the present study

| Name of primer | Sequence(5′-3′) | GenBank accession No. |
|----------------|-----------------|-----------------------|
| CcIRF2-F       | CAGATTCCGTGGATGCATG |                       |
| CcIRF2-R       | GTCTTCTAGATGACCTGGT |                       |
| CcIRF2 RT-F    | TGGACGACAGACGATACAGACAG |                       |
| CcIRF2 RT-R    | GCAGTGCCATCTCACCTCTTGG |                       |
| CcIRF2 ORF-F   | CAAAAGCTTTATGCCGAGGAATGCGT |                       |
| CcIRF2 ORF-R   | TCCCCGGGGCAAGCTTGAGGATTTGG |                       |
| CsS11-F        | CCGTGAGGTGACATCGTACA | AB012087              |
| CsS11-R        | TCAGGAACATTGCACTCTACTGCT | FN178457              |
| EPC-IFN-F      | CGCTAAGGTGGAGACAGGTTA |                       |
| EPC-IFN-R      | TTAGGTTCAATGTGCTCAGTCA |                       |
| EPC-PKR-F      | TGGAGACCTCGGCTCTGAGT | KM099176              |
| EPC-PKR-R      | TCCTGAGCTCGGCTCATGTA | KM099174              |
| EPC-Viperin-F  | AAAGACCTCTCGGACGCGCAAAGA |                       |
| EPC-Viperin-R  | CCTCTCGGAAATCTCAAGAAGCG |                       |
| EPC-ISG15-F    | ACCGATGCGAATCAACGAAAGCTC | KM099174              |
| EPC-ISG15-R    | CGTAACGCTTGGAGCTCAGTGAAT |                       |
| EPC-IRF3-F     | CGTGCACACACCATGCTGAGG | KJ027520              |
| EPC-IRF3-R     | ATCCAGATCTCCACCACCTCTTGT |                       |
| EPC-β-actin-F  | GCCGCTGACCTGACTGACTACCT | KF844250              |
| EPC-β-actin-R  | GCCACATAGCACGACTCCTCTTGG |                       |
### DBD (DNA binding domain)

| Species            | Sequence                      | Accession Numbers | Score |
|--------------------|-------------------------------|-------------------|-------|
| Cyprinus carpio    | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 69    |
| Ctenopharyngodon idella | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 69    |
| Paralichthys olivaceus | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 70    |
| Xenopus laevis     | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 70    |
| Gallus gallus      | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 70    |
| Mus musculus       | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 70    |
| Homo_sapiens       | MVPERMRMPSP VLEQISECK IPGLIMNKE KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 70    |

**Clustal_Consensus**

| Species            | Sequence                      | Accession Numbers | Score |
|--------------------|-------------------------------|-------------------|-------|
| Cyprinus carpio    | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 80    |
| Ctenopharyngodon idella | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 134   |
| Paralichthys olivaceus | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 134   |
| Xenopus laevis     | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 134   |
| Gallus gallus      | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 134   |
| Mus musculus       | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 134   |
| Homo_sapiens       | **** **.*.+:;:*******:;:******:*:;:***:*:* |                   | 134   |

**IAD2 (IRF associated domain 2)**

| Species            | Sequence                      | Accession Numbers | Score |
|--------------------|-------------------------------|-------------------|-------|
| Cyprinus carpio    | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 228   |
| Ctenopharyngodon idella | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 258   |
| Paralichthys olivaceus | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 258   |
| Xenopus laevis     | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 258   |
| Gallus gallus      | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 258   |
| Mus musculus       | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 258   |
| Homo_sapiens       | EQVTSDLLY PLQISVSYY QEDTDSDAS EDS---**** KRIQIPPMH AARGHVEK DAPLPMWAH ITGYKQGID     |                   | 258   |

**Clustal_Consensus**

| Species            | Sequence                      | Accession Numbers | Score |
|--------------------|-------------------------------|-------------------|-------|
| Cyprinus carpio    | *, .: ;: *: :*: * :*: * :*: * :*: * |                   | 290   |
| Ctenopharyngodon idella | *, .: ;: *: :*: * :*: * :*: * :*: * |                   | 328   |
| Paralichthys olivaceus | *, .: ;: *: :*: * :*: * :*: * :*: * |                   | 328   |

**Fig. 1** Multiple sequence alignment of IRF2 protein sequences from different species. Identical (*) and similar (: or .) residues are indicated. The DBD and IAD are indicated by black lines. Five tryptophan (W) residues are boxed in red. The GenBank accession numbers of the genes are listed in Table 2.
The expression pattern of CcIRF2

The expression of CcIRF2 in eleven tissues of healthy common carp was examined using Real-time PCR method, including the liver, spleen, head kidney, foregut, hindgut, gills, gonad, skin, muscle, buccal epithelium, and brain. The results showed that CcIRF2 was expressed in all examined tissues, with higher expression in the gills, spleen and brain (Fig. 3).

CcIRF2 expression in response to poly (I:C) and A. hydrophila stimulation in vivo

To determine the immune function of CcIRF2, CcIRF2 expression was examined in several immune-related tissues of common carp after viral or bacterial challenge. Upon poly (I:C) stimulation, the expression of CcIRF2 was increased and peaked at 3 hpi in the liver (21.5-fold), spleen (29.7-fold), head kidney (12.4-fold), foregut (26.3-fold) and hindgut (23.4-fold), and peaked at 6 hpi in the skin (34.2-fold) (Fig. 4). In the fish treated with formalin-killed A. hydrophila, CcIRF2 expression was upregulated in the head kidney (4.5-fold) and hindgut (6.5-fold) at 6 hpi and in the foregut (13.7-fold) at 24 hpi, while decreased in the spleen at 12 hpi (Fig. 5).

Expression of CcIRF2 upon poly (I:C), LPS and PGN stimulation in vitro

To determine the pathogen-associated molecular patterns (PAMPs) recognized by CcIRF2, the PBLs and HKLs of common carp were stimulated with poly (I:C), LPS, PGN or flagellin, and the expression of CcIRF2 was detected by Real-time PCR. The results showed that CcIRF2 expression in the PBLs was upregulated by poly (I:C) (4.7-fold), PGN (2.9-fold) and flagellin (1.6-fold) at 12 h but not changed by LPS (Fig. 6). In HKLs, CcIRF2

Table 2: Protein length and GenBank accession numbers of the IRF2 family members

| Species                        | Protein length | GenBank accession No. |
|--------------------------------|----------------|-----------------------|
| Cyprinus carpio                | 334            | MW559072              |
| Ctenopharyngodon idella        | 326            | AFV99156              |
| Danio rerio                    | 314            | NP_001307117          |
| Scophthalmus maximus           | 330            | AOV86412              |
| Paralichthys olivaceus         | 330            | ADZ96216              |
| Salmo salar                    | 343            | AC133066              |
| Xenopus laevis                 | 347            | NP_001088726          |
| Gallus gallus                  | 348            | NP_990527             |
| Mus musculus                   | 349            | AA10667               |
| Homo sapiens                   | 349            | CAG3335               |
| Pinctada maculata              | 350            | AFZ76969              |
| Branchiostoma belcheri tsingtauense | 338    | AJA02097              |
was induced by poly (I:C), LPS, PGN at 24 h (1.4-, 2.4-, and 2.6-fold) and flagellin at 12 h (1.2-fold) (Fig. 7).

Regulatory role of CcIRF2 in the IFN signalling
To investigate the role of CcIRF2 in the IFN signalling pathway, the gene expression of IFN, three ISGs (PKR, Viperin and ISG15) and IRF3 was detected after overexpression of CcIRF2 in EPC cells. The results showed that the expression level of these genes was significantly reduced, with 49, 54, 86, 66 and 15% of the level in control cells, respectively (Fig. 8).

Regulation of NF-κB by CcIRF2
To determine the role of CcIRF2 in the activation of NF-κB, dual-luciferase reporter assay was performed in 293 T cells. The results showed that MyD88 or TRIF could activate NF-κB, and CcIRF2 enhanced MyD88- or TRIF-induced NF-κB activity (Fig. 9).

Discussion
IRFs, which were originally described as transcription factors induced by IFN, play a variety of roles in immune response, immune system development and cell growth [4, 34–36]. With the development of the fishery economy, an increasing number of studies on IRFs in bony fish have been carried out. In the present study, the full-
Fig. 5 Expression analysis of CcIRF2 in response to A. hydrophila challenge in vivo. Total RNA was extracted from the spleen (a), head kidney (b), foregut (c) and hindgut (d) tissues at 0 (as a control), 3, 6, 12, 24, 48 and 72 hpi for real-time PCR. The expression was normalized using that of the 40S ribosomal protein S11 (n = 3, mean ± SD, *P < 0.05)

Fig. 6 Expression levels of CcIRF2 in PBLs upon different stimulation. Expression of CcIRF2 in the PBLs induced by poly (I:C) (a), LPS (b), PGN (c) or Flagellin (d). The expression levels were normalized using the 40S ribosomal protein S11 (mean ± SD, *P < 0.05)
Fig. 7 Expression levels of CcIRF2 in HKLs upon different stimulation. Expression of CcIRF2 in the HKLs induced by poly (I:C) (a), LPS (b), PGN (c) or Flagellin (d). The expression levels were normalized using the 40S ribosomal protein S11 (mean ± SD, *P < 0.05).

Fig. 8 Effect of CcIRF2 overexpression on expression of the IFN (a), PKR (b), Viperin (c), ISG15 (d) and IRF3 (e) genes in EPC cells. The expression levels were normalized to β-actin (mean ± SD, *P < 0.05).
length CcIRF2 cDNA was cloned from common carp, which encodes 334 amino acid residues. Notably, IRF2 encodes 349 amino acid residues in human and mouse, 348 in chicken and 347 in xenopus, but fewer than 345 in fish, reflecting the important role of IRF2 in the process of biological evolution and possible differences in protein function of IRF2 from different species.

Both mammalian and fish IRFs contain a DBD in the N-terminal region, which is responsible for binding the same ISRE/IRF-E sequences on target genes [37]. The CcIRF2 protein sequence was compared with that from pearl oyster, amphioxus, African clawed frog, chicken, mouse and human. The results showed that the DBD was relatively conserved, and the DBD in all species except amphioxus contained six tryptophan residues, suggesting that the function of the IRF2 molecule may be relatively evolutionarily conserved. There are two types of IRF C-termini: IAD1 was originally found in IRF8 and is present in all members of the IRF family except IRF1 and IRF2, while IAD2 exists in only IRF1 and IRF2 [38]. In the present study, the C-terminus of CcIRF2 contains the IAD2 domain.

The known IRF2 protein sequences from different species were used to construct an evolutionary tree using the neighbour-joining method. In the tree, IRF2s of teleosts, amphibia, bird and mammals are on a branch, while IRF2s of lamellibranchia and appendicularia are separate. The 11 members of the fish IRFs family are divided into four clades: the IRF1 subfamily, consisting of IRF1, IRF2 and IRF11; the IRF3 subfamily, consisting of IRF3 and IRF7; the IRF4 subfamily, consisting of IRF4, IRF8, IRF9 and IRF10; and the IRF5 subfamily, consisting of IRF5 and IRF6. CcIRF2 is closely related to grass carp in the branch containing fish species, which belongs to the IRF1 subfamily.

IRF2 is widely expressed in a variety of tissues in bony fish, such as E. coioides and O. mykiss [6, 10]. In the present study, the expression pattern of CcIRF2 in 11 tissues of healthy common carp was investigated, which was expressed in all tissues, with higher expression level in the gills, spleen and brain. Concordant with these results, the expression of IRF2 is highest in the gills or spleen of P. spatula, M. amblycephala, C. argus and C. idella [8, 12, 15]. In fish, the spleen and gills are important systemic and mucosal immune organs, respectively, and play a key defensive role against pathogen invasion, suggesting that CcIRF2 plays an important role in the immune system of fish. Unexpectedly, the expression level of IRF2 in the brain of common carp was also high, implying that IRF2 may be involved in regulating the nervous system of fish.

The previous studies in fish have shown that IRF2 can be induced by poly (I:C), including mandarin fish, paddlefish, rainbow trout and snakehead [6–8, 12], and the expression level of IRF2 in the liver, spleen and head kidney of half-smooth tongue sole was also significantly increased after viral stimulation [39]. In the present study, after poly (I:C) stimulation, the expression of CcIRF2 in all tissues increased and peaked at 3 h or 6 h, and the expression of CcIRF2 in the skin and spleen increased to the maximum level, suggesting that IRF2 may play an important role in the early immune response of common carp to viruses. After stimulation by A. hydrophila, the expression of CcIRF2 in the head kidney, foregut and hindgut was increased, but significantly decreased in the spleen. Similar to the results, after stimulation with A. hydrophila, the expression of IRF2 was increased in the spleen and head kidney but significantly decreased in the gills and intestines of blunt-nose bream. Therefore, the antibacterial immune process in which IRF2 is involved may be tissue-specific.

Similar to the in vivo results, poly (I:C) induced an increase in CcIRF2 expression in PBLs and HKLs. However, there was no significant change in CcIRF2 expression in PBLs after LPS stimulation. Studies in mammals have shown that LPS can induce the expression of IRF genes in B cells, T cells, macrophages and dendritic cells [40, 41]. However, fish can resist the toxic effects of LPS [42], and lack costimulatory molecules (such as MyD2 and CD14) produced by TLR4 during LPS-induced immune activation [43]. Therefore, the unchanged expression of CcIRF2 after LPS stimulation may be due to the different mechanism of LPS recognition between fish and mammals.

Mammalian IRF2 is unrelated to the production of IFN but is involved in the transcriptional regulation of
downstream genes such as histone H4, IL-7, IL-12, iNOS and MHC class I molecules [44–48]. The previous studies have shown that mammalian IRF2 plays a role in inhibiting transcriptional regulation due to its competition with IRF1 [49], and amphioxus IRF2 can compete with other IRFs for the ISRE-binding site in the nucleus, thus IRF2 is considered a negative regulator of the transcription process [50]. In this study, the overexpression of IRF2 in common carp inhibited the expression of IFN, PKR, Viperin, ISG15 and IRF3 in EPC cells, so CcIRF2 may also negatively regulate the expression of IFN and related factors. However, the dual-luciferase reporter assay showed that CcIRF2 activated the NF-κB signalling pathway and promoted both MyD88-mediated and TRIF-mediated NF-κB production. Mammalian IRF2 was reported to regulate NF-κB activity by regulating the subcellular localization of NF-κB [51], and IRF2 in pearl oyster can also activate the NF-κB signalling pathways [52]. Thus, IRF2 belongs to a class of multifunctional transcription factors whose specific roles in transcriptional inhibition or activation depend on cell type and inflammatory state [4, 53, 54].

Conclusions
In the present study, the full-length cDNA of the IRF2 gene was identified from common carp, and its effects on defence against pathogen invasion and regulation of the IFN response were investigated in vivo and in vitro. The results indicate that CcIRF2 participated in antiviral and antibacterial immunity and negatively regulated the IFN response, which provide a new insight into the regulation of IFN system in common carp, and are helpful for the prevention and control of infectious diseases in carp farming.

Abbreviations
IFN: interferon; IRF: interferon regulatory factor; ISG: IFN-stimulated gene; DBD: DNA-binding domain; NLS: nuclear localization signal; IAD: IRF associated domain; poly (I:C): polyinosinic-polycytidylic acid; LPS: lipopolysaccharide; EPC: epithelioma papulosum cyprinid; RACE: rapid amplification of the cDNA ends; SMART: simple modular architecture research tool; PBL: peripheral blood leukocyte; HKL: head kidney leukocyte; PGN: peptidoglycan; ORF: open reading frame; ANOVA: one-way analysis of variance; UTR: untranslated region; hpi: hour post injection; ISRE: IFN signalling pathway and promoter assay showed that

Acknowledgments
Not applicable.

Authors’ contributions
H.L. and G.Y. conceived and designed the experiments. X.C., Y.Z., R.L., L.Z. and F.Z. performed the experiments. S.S. and L.A. analyzed the data. H.L. and X.C. wrote the paper. All authors reviewed the manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article is available in the GenBank (https://www.ncbi.nlm.nih.gov/nuccore/MW559072) and the accession number is MW559072.

Declarations
Ethics approval and consent to participate
The protocol was approved by the Animal Experimental Ethics Committee of Shandong Normal University, and all methods were performed in accordance with the relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

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