Molecular identification and transcriptional regulation of porcine IFIT2 gene

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Received: 6 October 2017 / Accepted: 2 April 2018 / Published online: 6 April 2018
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Abstract
IFN-induced protein with tetratricopeptide repeats 2 (IFIT2) plays important roles in host defense against viral infection as revealed by studies in humans and mice. However, little is known on porcine IFIT2 (pIFIT2). Here, we performed molecular cloning, expression profile, and transcriptional regulation analysis of pIFIT2. pIFIT2 gene, located on chromosome 14, is composed of two exons and have a complete coding sequence of 1407 bp. The encoded polypeptide, 468 aa in length, has three tetratricopeptide repeat motifs. pIFIT2 gene was unevenly distributed in all eleven tissues studied with the most abundance in spleen. Poly(I:C) treatment notably strongly upregulated the mRNA level and promoter activity of pIFIT2 gene. Upstream sequence of 1759 bp from the start codon which was assigned +1 here has promoter activity, and deltaEF1 acts as transcription repressor through binding to sequences at position −1774 to −1764. Minimal promoter region exists within nucleotide position −162 and −126. Two adjacent interferon-stimulated response elements (ISREs) and two nuclear factor (NF)-κB binding sites were identified within position −310 and −126. The ISRE elements act alone and in synergy with the one closer to start codon having more strength, so do the NF-κB binding sites. Synergistic effect was also found between the ISRE and NF-κB binding sites. Additionally, a third ISRE element was identified within position −1661 to −1579. These findings will contribute to clarifying the antiviral effect and underlying mechanisms of pIFIT2.

Keywords Pig · IFIT2 · Cloning · Expression · Poly(I:C) · Promoter

Introduction
Host innate immune system secrets type I interferon (IFN) upon the detection of viruses, which, in turn, triggers the induction of hundreds of IFN-stimulated genes (ISGs). ISGs can block virus at multiple stages including modulation of viral entry into cells, translation initiation, propagation, and spread [1, 2]. Among the first ISGs to be discovered and cloned is the interferon-induced protein with tetratricopeptide repeats (IFITs) family composed of IFIT1, 2, 3, 5, and several pseudogenes and IFIT-like genes [3]. IFIT proteins are characterized by multiple repeats of tetratricopeptide repeat (TPR) helix-turn-helix motifs which are involved in numerous protein–protein interactions during various physiological and pathological process including translation initiation, cell migration, proliferation, double-stranded RNA signaling, and virus replication [4].

IFIT2, one of heavily studied family member, plays important roles in blocking viral infection. It is responsible for protecting mice from pathogenesis caused by the rhabdovirus Vesicular stomatitis virus [5, 6] and Rabies virus [7], the coronavirus mouse hepatitis virus [8], and the respirovirus Sendai virus [9]. Human IFIT2 is found to limit Hepatitis B virus replication in cultured cells [10]. It also restricts West Nile virus infection and pathogenesis in various human tissues in a cell type-specific manner, and contributes to the induction or magnitude of immune responses [11].
The extensive antiviral effect of IFIT2 in human and mouse suggests its potential in protecting pig from viral disease. However, little is known on porcine IFIT2. In the present work, we characterized the sequence, structure, and tissue expression profile of IFIT2 gene. To preliminarily explore its antiviral properties, in vitro experiments were conducted after treatment with viral analog, poly(I:C), a synthetic double-stranded RNA and has been extensively used to mimic viral infection [12, 13]. Moreover, the promoter activity and major cis-acting elements were identified as well. The results contribute to further revealing the antiviral effect and underlying mechanisms of IFIT2, which will be helpful in breeding pigs with resistance to viral diseases.

Materials and methods

Animals, nucleotide acid isolation

Tissues including stomach, kidney, bladder, spleen, lung, heart, liver, large intestine, small intestine, muscle, and lymph were collected immediately after the pigs were slaughtered, snap frozen in liquid nitrogen and stored at −70 °C. The protocol of animal treatment was according to the guidelines of the Ministry of Science and Technology of China [14]. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), treated with DNase I to remove the potential DNA contamination, and diluted into 1 µg/µl for future use. Genomic DNA was isolated from ear tissue using phenol/chloroform.

cDNA cloning

Based on EST, electronic sequences in pigs and orthologs in other species including human, mouse, dog, etc, one primer pair (Forward: 5′-aggaggatttctgaagagcac-3′; Reverse: 5′-attttttgaatcaactcgg-3′) were designed for cloning porcine IFIT2 cDNA. Reverse transcription (RT) was performed using PrimeScript® RT reagent kit (Perfect Real Time) (Takara, Dalian, China) with oligo(dT) primer and 1 µg total RNA as template. PCR reactions were conducted in a final volume of 25 µl containing 1×PCR buffer, 200 µM each dNTPs, 200 nM of each primer, 1 U Taq DNA polymerase (Takara), and 1 µl cDNA. PCR conditions were as follows, 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and 72 °C for 7 min. The PCR products were sub-cloned into pMD18-T (Takara) vector and sequenced by the Beijing Genomics Institute (BGI, Shenzhen, China). Sequence analysis was performed with the DNAMAN package (version 5.2.2) and the BLAST program at the National Center for Biotechnology Information (NCBI) website.

Real-time quantitative PCR

Expression profile of pIFIT2 gene in tissues and that induced by poly(I:C) was analyzed using real-time quantitative PCR (qPCR). PK-15 cells were cultured as described previously [15] and stimulated with different poly(I:C) concentrations for 12 hours (h). cDNA from cells or tissues were synthesized as described above. qPCR was performed on Applied Biosystems 7500 instrument with SYBR Premix Ex Taq™ II (Takara) according to the manufacturer’s protocol. Each reaction was run in 10 µl total volume in triplicate. The relative mRNA abundance of target genes was calculated using 2−ΔΔCt method with β-actin as reference [16]. The primer pair for pIFIT2 was as follows: Forward: 5′-gacggcagagaatgaaatgtg-3′; Reverse: 5′-gcaggcgagatttctgaagagcac-3′.

Firefly luciferase reporter construct

Sequence between nucleotide (nt) position −1838 and −46 of pIFIT2 gene was amplified from genomic DNA. The resulting products were first sub-cloned into pMD18-T (Takara) vector, and then ligated upstream of the firefly luciferase reporter gene in the pGL3-basic vector (Promega, Madison, WI) using the enzymes, KpnI and XhoI, which were introduced into the end of products by primers. At the same time, a series of 5′ truncated fragments at nt position −1759, −1695, −1661, −1579, −1499, −1397, −1146, −894, −654, −389, −310, −225, −162, and −126 with a common 3′-terminus at −46 bp were cloned into the pGL3-basic vector. In this report, the first nucleotide of coding sequence (CDS) was assigned position +1. Recombinant pGL3 plasmid obtained was named according to the name of forward primer used for amplification of inserted fragment, such as pGL3-A, pGL3-B, etc. The primers were given in Table 1.

Meanwhile, fragments absent of predicted deltaEF1 binding site, ISRE element, and NF-kB binding site were generated by overlap extension PCR as described previously [15]. Briefly, the deletion was first introduced into the ends of overlapping fragments by PCR with primers absent of the target site, respectively. And then the resulting products were spliced into a bigger one to introduce the deletion into the inner. The fragments obtained were inserted into the pGL3-basic vector (Promega), as described above, to construct mutant type reporter genes. All the reporter gene constructs were verified by DNA sequencing (BGI, Shenzhen, China). The primers used for
mutation were given in Table 2, and experiment project for site-directed deletion was given in Table 3.

Dual-luciferase reporter analysis

Each reporter gene construct was cotransfected into PK-15 cells with Renilla luciferase reporter plasmid (pRL-TK) (Promega, Madison, WI) using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s protocol. At 24 h after transfection, the cells were collected and firefly and Renilla luciferase activities were measured with the Dual-Glo Luciferase Assay System (Promega). Relative luciferase activity was calculated as a ratio of firefly luciferase to Renilla luciferase. For poly(I:C) induction analysis, the cells were further treated in varied concentrations and time periods as indicated under “Results” before luciferase activity was measured.

Results

cDNA cloning and sequence analysis

The cDNA obtained was 1558 bp in length containing the complete CDS of 1407 bp, a 3′ untranslated region (UTR) of 80 bp, and a 5′ UTR of 71 bp. The sequence similarities of pIFIT2 CDS with the homologs in human (NM_001547) and mouse (NM_008332.3) were 81.36 and 70.89%, respectively. Conceptual translation predicted that pIFIT2 protein, composed of 468 amino acids, had a theoretical molecular mass of 54 kDa and an isoelectric point of 6.92.

The pIFIT2 gene was mapped to chromosome 14 using blat program (http://genome.ucsc.edu/cgi-bin/hgBlat). It is composed of two exons with the first one providing 5′ UTR and 5 nt of the CDS, and the second one containing the remaining part of the CDS and 3′ UTR. The gene structure of pIFIT2 is identical to the homolog in human, somewhat different from murine IFIT2 which has an extra short upstream exon (Fig. 1). A default motif search in SMART database (http://smart.embl-heidelberg.de/) revealed the presence of three TPR motifs in porcine and human IFIT2 proteins (Table 4), respectively, however no TPR was predicted in murine IFIT2 with the same method.

To gain insight of the evolutionary relationship between pIFIT2 and the homologs in other vertebrates, a phylogenetic

| Name  | Sequence (5′–3′) | Position |
|-------|-----------------|----------|
| deltaEF1-F | AACTGATGCAGCCCTTCCTCTCATT | −1789 |
| deltaEF1-R | CTTCTGAGATGAGGAAAGGCTGCAT | −1749 |
| ISREIIF-F | CTTCTCACTGAGTCATAGAAAGCCGTTAGGAG | −1639 |
| ISREIIF-R | CTCACAGGTTTCTATATCTACTGAGAGTTC | −1596 |
| ISREIIF-F | GAGTACTGAAATCCTTCCTTTTCTTAAAG | −184 |
| ISREIIF-R | GCCATGAGAAGAAGGGAATGGAATGGCAGTAC | −138 |
| ISREIIF-F | AGATGCTGCGACTTACTTACGTTC | −185 |
| ISREIIF-R | GAAAGGAGAAATGGAATCCTGAGATCTCCT | −144 |
| DIS-F | AGAGTACTGCTTTCTCTAAAGCCTG | −185 |
| DIS-R | ACAGCTTGGAGAAAGGCAGTACTC | −134 |
| NF-xBII-F | TCTTACTAAGATTAGTACTGCCC | −209 |
| NF-xBII-R | GCGAGTAGCTCTAATTCCTTAGAAGGCTG | −175 |
| NF-xBIII-F | CTCCCTTGTAGGCAGAAGAATCTATACTC | −303 |
| NF-xBIII-R | GAATTTTTTCTGCCATCAAGGAGGCC | −268 |
We found that IFIT2 gene only exists in mammals. The ancient IFIT2 gene originated from sorae and was divided into four subgroups, Rodentia A, Insectivora B, Rodentia B, and Insectivora C/Chiroptera. Rodentia B is the ancestor of euarchontoglires to which human being belongs, while Insectivora C/Chiroptera is the ancestor of laurasiatheria to which pig belongs. The evolution of IFIT2 gene in euarchontoglires and laurasiatheria tends to stable. The phylogenetic relationship revealed by the tree topology conforms to the taxonomic classification of the species used. The sequences have been submitted to the GenBank database under Accession No. JX070559.

Expression profiles

pIFIT2 gene was ubiquitously expressed in all 11 tissues analyzed with the most abundance in spleen, followed by liver, and heart. In the remaining tissues, including lung, kidney, stomach, muscle, lymph, large intestine, and small intestine, the relative mRNA level was much lower with the least abundance in bladder (Fig. 3).

The kinetics of transcriptional induction of pIFIT2 gene in response to poly(I:C) were analyzed with pGL3-B construct. The induction was in a time- and dose-dependent manner. The best induction time was for 8–12 h and the promoter activity was dropped when the time was for 24 h (Fig. 4a). Therefore, 12 h was used as induction time in the following experiments. The promoter activity was increased with the increasing of poly(I:C) concentration. It was induced slightly when the concentration was from 0.5 to 5 ng/ml, and sharply at a concentration of 10 ng/ml (Fig. 4b). At the same time, the response of endogenous pIFIT2 gene to poly(I:C) was measured with RT-qPCR method in PK-15 cells, and the similar results was obtained (Fig. 4c).

Table 3

| Elements | PCR 1 | PCR 2 | PCR 3 | Splicing PCR |
|----------|-------|-------|-------|--------------|
| ΔdeltaEF1 | A-F/deltaEF1-R | deltaEF1-F/P-R | | A-F/P-R |
| ΔISREIII | A-F/ISREIII-R | ISREIII-F/P-R | | B-F/P-R |
| ΔISREI | G-F/ISREI-R | ISREI-F/P-R | | G1-F/P-R |
| ΔISREII | G-F/ISREII-R | ISREII-F/P-R | | G1-F/P-R |
| ΔISREI-II | G-F/DIS-R | DIS-F/P-R | | G1-F/P-R |
| ΔNF-xBII | G-F/NF-xBII-R | NF-xBII-F/P-R | | G1-F/P-R |
| ΔNF-xBII-I | G-F/NF-xBII-R | NF-xBII-F/NF-xBII-R | NF-xBII-F/P-R | G1-F/P-R |
| ΔNF-xBII + ISREI-II | G-F/NF-xBII-R | NF-xBII-F/DIS-R | DIS-F/P-R | G1-F/P-R |
| ΔNF-xBII + ISREII | G-F/NF-xBII-R | NF-xBII-F/DIS-R | DIS-F/P-R | G1-F/P-R |
| ΔNF-xBII-II + ISREI-II | G-F/DIS-R | DIS-F/P-R | | G1-F/P-R |
| ΔNF-xBII + ISREI-II | G-F/DIS-R | DIS-F/P-R | | G1-F/P-R |

ISREI-II and NF-xBII-II indicate double deletion of ISREs and NF-xB binding sites, respectively, while NF-xBII-I + ISREI-II indicates that two ISRE elements and two NF-xB binding sites were deleted simultaneously. In regular PCRs 1, 2, and 3, cloning plasmid containing fragment from −1838 to −45 of pIFIT2 gene was used as template except for deleting NF-xBII-II + ISREI-II in which PCR products having NF-xBII-II deletion was used as template; in the following splicing PCRs, products of PCRs 1, 2 and 3, if have, were mixed equally and used as template.

Table 4

| Name         | Species          | GenBank No. | Polypeptide length | TPR 1a | TPR 2a | TPR 3a |
|--------------|------------------|-------------|--------------------|-------|-------|-------|
| Pig          | Sus scrofa       | JX_070559   | 468                | 51–84 | 94–127| 243–276|
| Human        | Homo sapiens     | NM_001547   | 472                | 51–84 | 172–208| 247–280|

*Position of TPR motifs in the polypeptide
Promoter identification

We transfected a series of luciferase reporter gene constructs containing pIFIT2 gene upstream region into PK-15 cells (Fig. 5a). The results showed that the construct pGL3-B directs highest level luciferase expression, while construct pGL3-A has little promoter activity. Also evident, the construct pGL3-G directs above background level luciferase expression. Additionally, significant difference in luciferase expression level was observed between the constructs pGL3-B and pGL3-C. These data indicate that there are basal promoter region between nt position −389 and −45, positive regulatory element between −1661 and −1397, and potent repressive element within −1838 to −1661.

Further analysis narrows the repressive element to nt position −1838 to −1759 and the positive regulatory regions to −1661 to −1579 (Fig. 5b, c). Additionally, significant differences in luciferase expression level were observed between constructs pGL3-G and pGL3-G1, pGL3-G1 and pGL3-G2, and between pGL3-G2 and pGL3-G3. No luciferase activity was measured for construct pGL3-G4. These data indicate that regulatory elements exist within the nt positions −389 and −126, and that the minimal promoter region exists within position −162 and −126 (Fig. 5d).

Identification of the major regulatory element

Using online program TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html) and Alibaba 2 (http://
www.gene-regulation.com/pub/programs/alibaba2/index.html), five sequence motifs, including two consecutive ISREs and nuclear factor (NF)-κB binding sites located at position −310 to −126, and another ISRE at position −1661 to −1579, were identified (Fig. 6). ISREI motif exactly matches the consensus sequence, 5′-AGT TTTT NNTTTC(C/T)-3′, whereas ISREII and ISREIII diverge at a few nucleotide positions. Additionally, one deltaEF1 binding sequence was present at position −1774 to −1764.

To test the functional significance of the candidate cis-acting elements we prepared mutant type reporter constructs with the sequences deleted. A total of one pGL3-A mutant, one pGL3-B mutant, and nine pGL3-G1 mutants were obtained. In each modified construct, the whole candidate sequence was deleted. DeltaEF1 binding site deletion restored the promoter activity strongly (Fig. 6a). The promoter activity of construct deleting ISREIII was 33.76% compared to its wild type counterpart (Fig. 6b).

The two ISRE motifs at position −310 to −126 acts alone and in synergy to activate the promoter activity, so do the NF-κB binding sites. The promoter activity was decreased strongly in the absence of ISREI (12.72% compared to the wild type counterpart) or ISREII (55.04%). The deletion of both elements produced a synergistic response in that only 3.89% activity was found compared to the wild type counterpart.

Similarly, the promoter activity was decreased with the deletion of NF-κBI (35.3%) or NF-κBII binding site (53.81%), and synergistic response (21.7%) existed in double deletion. Synergistic effects were also found between ISRE and NF-κB binding sites: when one of the NF-κB binding site and both ISRE elements were deleted simultaneously, the activity was only 1.94% (NF-κBI and both ISRE deleting) and 2.78% (NF-κBII and both ISRE deleting), respectively, compared to the wild type construct; when the four motifs at the

Fig. 3 Tissue expression profile of porcine IFIT2 gene. The relative expression level in bladder was used as 1. Each column represents the mean ± SE of triplicate determinations

Fig. 4 Induction kinetics of pIFIT2 by poly(I:C). PK-15 cells were induced with 10 ng/ml poly(I:C) for different time periods (a) or with different concentrations of poly(I:C) for 12 h (b) after transiently transfected with promoter construct pGL3-B for 24 h. The relative luciferase activity is displayed relative to the non-treatment control, which was used as 1. c Induction of endogenous pIFIT2 by poly(I:C). PK-15 cells were induced by poly(I:C) for 12 h. The relative mRNA level is measured with RT-qPCR method and displayed relative to the non-treatment control, which was used as 1. Data are representative of three individual experiments, each with three replicates. Each column represents the mean ± SE
Deletion of ISRE and/or NF-κB binding sites caused a strong decrease of poly(I:C)-induced promoter activity. The induction of pGL3-B construct deleting ISREIII by poly(I:C) was 71% compared to the wild type control. The promoter induction was also strongly reduced with the deletion of ISREI (28% compared to the wild type counterpart).
**A**

deltaEF1: -1774 TCAAGGTTGGCT -1764

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 promoter construct
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**B**

ISREIII: -1626 AGTTTCAATTCTCT -1613

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 promoter construct
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**C**

NF-κBII: -287 AGGAAATTCAC -277

NF-κBI: -195 CATTTCCCTTT -185

ISREII: -173 AATTCCTCCCC -162

ISREI: -161 AGTTTCAATTCTCT -149

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 promoter construct
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IFIT mRNA in untreated HT1080 or small intestine, it is undetectable or detectable at very low levels with western blotting method [18]. Also, there are no detectable levels of IFIT mRNA in untreated HT1080 cells and HEK293 cells using RNase protection assay [17]. The sensitivity of the methods used may be the reason for the differences between our results and others. The IFIT2 is induced by poly(I:C) in a time- and dose-dependent manner, and the induction pattern is similar to that of human IFIT2 gene whose mRNA level is induced strongly by poly(I:C) at 6 h, remained constant at 12 h, and dropped sharply between 12 and 24 h in HT1080 cells [17]. Studies have shown that IFIT genes are regulated in a cell type-, inducer-, and gene-specific manner [18–20]. These results indicate that IFIT2 is highly regulated and might have similar role in viral defense to that of homolog in human.

The upstream sequence of the IFIT2 can drive the expression of firefly luciferase, while the strength can be repressed almost completely if a 79 bp region (positions − 1838 to − 1759) further upstream is included (up to position − 1838). This indicates that the upstream region corresponds to a functional promoter, and that a potent repressive element exists between positions − 1838 and − 1759. Bioinformatic analysis has revealed common consensus sequence for transcription factor deltaEF1 between positions − 1838 and − 1759. DeltaEF1 factor, a zinc finger protein, is implicated in the regulation of a lot of viral and cellular genes with functionally diverse roles [21, 22]. Through binding directly to the promoters, it represses transcription of epithelial splicing regulatory protein 2 [23], E-cadherin [24] and Plakophilin 3 genes [25], and suppresses promoter activity of chicken infectious anaemia virus [26] as well. Here, we confirmed that deltaEF1 is also transcription repressor of IFIT2 gene. Additionally, the promoter activity was strongly enhanced when the fragment was truncated from nt position − 389 to − 310 at the 5’ end, indicating another repressor present here. No specific transcription factor binding site was predicted in the region using TFSEARCH program, while by using Alibaba 2 program, Id3 consensus sequence was identified. Id3 is a dominant-negative regulator of transcription by sequestering other TFs, thus making them unable to bind DNA [27, 28]. However, the promoter activity was not restored when the predicted motif was deleted partially or completely from construct pGL3-G (data not given). The results reveal that Id3 is not involved in the transcription of IFIT2, and there should be other factors regulating IFIT2 expression.

The ISRE is essential for IFN inducibility of ISGs. Here, we confirmed that there are two adjacent ISREs in IFIT2, as its counterparts in human and mouse [17, 18]. These two elements act synergistically with ISREI closer to the start codon, having more strength. Furthermore, a third functional ISRE element was identified in the upstream of IFIT2 promoter. To our knowledge, this is not reported previously for other IFIT promoters. As we use different reporter genes to confirm the potential elements, ISREIII and ISREII, we cannot reveal which one is the strongest in the induction

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**Discussion**

Porcine IFIT2 should be an important molecule in innate antiviral immune responses, as revealed by studies in human and mice. Elucidation of its role and regulatory mechanism underlying expression and activation will contribute to preventing viral infection and pathogenesis in pigs, and to breeding pigs resistant to viral diseases. Here the existence of IFIT2 gene is confirmed using molecular biology techniques for the first time, and its expression profile in tissues of 3 distinct in structure [17, 18]. We showed that IFIT2 has the same structure as its counterpart in human: both of them are composed of two exons and have three confidently predicted TPR motifs. The sequence similarity of IFIT2 between human and pig is high (81.36 and 72.30% at the CDS and amino acid level, respectively). Additionally, phylogenetic analysis, revealed an evolutionary relationship among mammals. These data provide an evidence that IFIT2 is evolutionally conserved. Sequence and structure are the basis for the function of a given gene. We thus expect similar roles of IFIT2 gene in viral infection and pathogenesis in pigs.

RT-qPCR revealed that IFIT2 transcripts were present in all tissues studied with different amounts. The expression of murine IFIT2 is strongly induced in response to type I IFNs, poly(I:C), and infection by a lot of viruses, while in untreated tissues including spleen, lung, kidney, liver, colon, and small intestine, it is undetectable or detectable at very low levels with western blotting method [18]. Also, there are no detectable levels of IFIT mRNA in untreated HT1080 or ISREII (52%). Similarly, pGL3-G1 construct absent of NF-κB binding site showed a strong decrease in transcripational response to poly(I:C), as well: the induction was 38 and 65%, respectively, compared to the wild type counterpart. The synergistic effects were also found between both ISRE elements or NF-κB binding sites. When the four motifs in the region were deleted simultaneously, the promoter was hardly response to poly(I:C) (Fig. 7).
is displayed relative to the non-treatment control, which was used as a µg/ml level of poly(I:C) concentration [29]. This may be correlated with the existence of ISREIII.

The role of ISRE motif in the transcriptional activation of ISGs is critical and well documented, while other transcription factors involved in regulating ISG expression remain to be clarified. NF-κB, a transcription factor family including p50, p52, p65/RelA, RelB, c-Rel, and v-Rel, regulates the expression of genes involved in antiviral immune response by binding to cis-acting sites in the promoters. The induction of murine Ifit1 and Isg15 was lower by type I IFN in NF-κB knock out fibroblasts than in wild-type cells, while the induction of Mx1 and Nmi was at much higher levels, indicating that NF-κB plays a selective and distinct role in regulating the expression of ISGs and the induction of antiviral activity [30]. Further analysis revealed that p50 directly binds to the promoter of Mx1 gene and thereby refrains its induction [31]. We demonstrated here that NF-κB binding sites play a positive role in the activation of pIFIT2, which is similar to the effects of NF-κB on Ifit1 and Isg15 induction. Further experiment needs to be performed to identify which member of NF-κB family binding to the cis-acting element. Nevertheless, we identified several important cis-acting elements in the promoter region of pIFIT2, which will contribute to clarifying the mechanism underlying its transcriptional regulation.

Acknowledgements This work was supported by the Scientific Research Foundation of Heilongjiang Provincial Education Department (12541014) and Foundation for Improving Innovative Capability of Scientific Institutions, Heilongjiang (YC2016D001).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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