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Grouper IFIT1 inhibits iridovirus and nodavirus infection by positively regulating interferon response

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ABSTRACT

Interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), one of the interferon stimulated genes (ISGs), is strongly induced by type I interferon (IFN), double-stranded RNAs and virus infection. To investigate the actions of fish IFIT1 in response to virus infection, we cloned an IFIT1 homolog from orange spotted grouper (EcIFIT1) and clarified its function in this study. The full-length cDNA of EcIFIT1 is 1839 bp, which is composed of 436 amino acid (aa) residues, with 77.8% and 22.8% identity to IFIT1 homolog of yellow perch (*Perca flavescens*) and humans (*homo sapiens*), respectively. Sequence alignment analysis showed that EcIFIT1 contained three tetratricopeptide repeats (TPRs). Tissue distribution analysis indicated that EcIFIT1 was abundant in intestine, spleen, liver, and heart. Moreover, EcIFIT1 was significantly up-regulated by Singapore grouper iridovirus (SGIV) or red-spotted grouper nervous necrosis virus (RGNNV) infection, and polyinosinic-polycytidylic acid (poly I:C) or lipopolysaccharide (LPS) treatment *in vitro*. Under fluorescence microscopy, EcIFIT1 was found to localize throughout the cytoplasm in transfected cells. EcIFIT1 overexpression significantly suppressed the replication of SGIV and RGNNV, demonstrated by decreasing the cytopathic effect (CPE) severity, viral gene transcription and the virus titers. Further studies showed that the ectopic expression of EcIFIT1 increased the transcription level of IFN related molecules, including IFN regulatory factor (IRF) 3, IRF7, IFN stimulated gene (ISG) 15 and myxovirus resistance gene (MX) I. Meanwhile, the expression levels of pro-inflammation cytokines were differently regulated by the ectopic expression of EcIFIT1. In addition, flow cytometry analysis suggested that EcIFIT1 overexpression affected cell cycle progression by mediating S/G2 transition. Taken together, our results indicated that EcIFIT1 might exert antiviral function against fish virus by up-regulating interferon response or affecting cell cycle.

1. Introduction

Interferons (IFNs) which act as secreted ligands of specific cell surface receptors, are triggered by various pathogens, such as viruses, bacteria, fungi and parasites, to active Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway and up-regulate the transcription of numerous IFN-stimulated genes (ISGs) [1,2]. It has been reported that many ISGs, including protein kinase R (PKR) [3], viperin [4,5], tetherin [6], tripartite motif containing proteins (TRIMs) [7–10], IFN-induced transmembrane proteins (IFITMs) [11,12], ISG15 [13] and IFN-induced protein with tetratricopeptide repeats (IFITs) [14,15] showed direct or indirect antiviral activity at different stages in the life cycle of viruses [16].

IFITs, a group of the identified ISGs, are strongly induced by type I IFN, double-stranded RNAs and virus infection [17,18]. To date, IFITs have characterized four members in humans, namely IFIT1/ISG56, IFIT2/ISG54, IFIT3/ISG60, and IFIT5/ISG58, which all contain multiple tetratricopeptide repeats (TPRs) known to mediate protein-protein interactions through scaffolds formed among tandem TPR [15]. A growing number of reports have revealed that IFIT1 has antiviral activity against both DNA and RNA virus, such as Japanese encephalitis virus (JEV) [19], parainfluenza virus (PIV) type 5 [14], hepatitis C virus (HCV) [20,21], human cytomegalovirus (HCMV) [22], human papillomaviruses (HPV) [23], lymphocytic choriomeningitis virus (LCMV) and West Nile virus (WNV) [24] via different mechanisms. For example, IFIT1 was found to bind eukaryotic initiation factor (eIF) 3 to inhibit

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viral protein translation [25]. In addition, IFIT1 was demonstrated to suppress the replication of HPV by binding to the viral protein E1 [23]. Moreover, many experiments showed that IFIT1 specifically recognized a 5′-triphosphate group (PPP-RNA) which is a molecular signature that distinguishes it from host RNA, to inhibit viral RNA replication [26–28]. Although there are many studies in elucidating the antiviral effects of IFIT1 against viruses in mammals, only limited literatures focused on the roles of IFIT1 in fish, including tongue sole (*Cynoglossus semilaevis*) [29], crucian carp (*Carassius auratus*) [30], large yellow croaker (*Larimichthys crocea*) [31], and olive flounder (*Paralichthys olivaceus*) [32].

Groupers, *Epinephelus* spp., are economic fish species that are widely cultured in China and Southeast Asian countries. In recent years, the emergence of virus diseases which caused by Singapore grouper iridovirus (SGIV) and red spotted grouper nervous necrosis virus (RGNNV) have caused heavy economic losses in the grouper aquaculture industry [33–35]. To explore the immune defense mechanisms of grouper, many immune regulatory molecules, such as ISG15 [13], mitochondrial antiviral signaling protein (MAVS) [36], IFN regulatory factor (IRF) 3 [37], IRF7 [38], melanoma differentiation-associated gene (MDA) 5 [39], cholesterol 25-hydroxylase (CH25H) [40] and viperin [5] were identified and characterized during virus infection. However, the detailed function of most ISGs in response to grouper virus infection still remained uncertain.

In this study, we cloned an IFIT1 homolog from orange spotted grouper (EcIFIT1) and detected the subcellular localization of EcIFIT1. Besides, the antiviral effects of EcIFIT1 during SGIV and RGNNV replication were investigated. Meanwhile, the roles of EcIFIT1 on host IFN immune, proinflammatory response and cell cycle were examined. Our data will shed lights on understanding the roles of fish EcIFIT1 against virus infection.

2. Materials and methods

2.1. Cells and virus

Grouper spleen (GS) cells used in this study were grown at 28 °C in Leibovitz's L15 medium (Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA) [41]. The virus stocks of SGIV and RGNNV were prepared in GS cells and grouper brain (GB) cells, respectively, and then were maintained at −80 °C.

2.2. Cloning of EcIFIT1

Based on the EST sequences of EcIFIT1 from grouper spleen transcriptome [42], we cloned the full length open reading frame (ORF) of EcIFIT1 using the primers listed in Table 1 by PCR and obtained the 5′ and 3′ ends of EcIFIT1 by the rapid amplification of cDNA ends (RACE) PCR using SMARTer® RACE 5′/3′ Kit (Clontech). The sequence of EcIFIT1 was analyzed by the BLAST program (http://www.ncbi.nlm.nih.gov/blast), and the conserved domains were predicted using the SMART program (http://smart.embl-heidelberg.de/). In addition, multiple sequences alignment and the Neighbor-joining (NJ) phylogenetic tree was created with the MEGA 6.0 software and the data was edited using the GeneDoc program.

2.3. Expression profiles of EcIFIT1

Orange-spotted groupers, *E. Coioides* (50–60 g) used in the study were purchased from Hainan Province, China, and maintained in a laboratory recirculating seawater system before use. The tissue distribution pattern of EcIFIT1 from 9 tissues, including liver, spleen, kidney, head kidney, brain, intestine, skin, muscle and heart were determined by quantitative real-time PCR (qPCR) as described in the following.

To examine the expression changes of EcIFIT1 in response to fish virus infection and pathogen-associated molecular pattern (PAMP) molecules, GS cells were seeded in 24-well plates overnight, and infected with SGIV or RGNNV at a multiplicity of infection (MOI) of 2.0, transfected with 200 ng polyinosinic-polycytidylic acid (poly I:C) or treated with 4 μg/mL lipopolysaccharide (LPS) as described previously [40]. Briefly, SGIV or RGNNV infected cells were collected at 0, 3, 6, 18, 24, 30, 42 h post-infection (h.p.i.) and poly I:C or LPS treated cells were harvested at 0, 4, 8, 12, 24, 36 h for further qPCR analysis.

Table 1
Primers used in this study.

Primer names	Sequence (5′-3′)
5′-EcIFIT1-R1	CCTCCAGCCCTGTTTCAC
5′-EcIFIT1-R2	CGCTGGGCGTCTTCTTT
3′-EcIFIT1-F1	TGGAGGCTGACATCTTGG
3′-EcIFIT1-F2	GTGAGTTAGCCAGAAAGGTTT
EcIFIT1-3HA-BamHI-F	CGCGGATCCATGATGAGTGCTGCTCAGAG
EcIFIT1-3HA-EcorI-R	CCGGAATTCCTATGGCTCTTGCAGATTTG
EcIFIT1-C1-EcorI-F	CCGGAATTCTATGATGAGTGCTGCTCAGAG
EcIFIT1-C1-BamHI-R	CGCGGATCCTGGCTCTTGCAGATTTGCC
EcIFIT1-RT-F	ATTTGGCAGAGGAGGCT
EcIFIT1-RT-R	CTTTGCTTTGGGCGACT
Actin-RT-F	TACGAGCTGCCTGACGGACA
Actin-RT-R	GGCTGTGATCTCCTTCTGCA
MCP-RT-F	GCACGCTTCTCTCACCTTCA
MCP-RT-R	AACGGCAACGGGAGCACTA
VP19-RT-F	TCCAAGGGAGAAACTGTAAG
VP19-RT-R	GGGGTAAGCGTGAAGAC
CP-RT-F	CAACTGACAACGATCACACCTTC
CP-RT-R	CAATCGAACACTCCAGCGACA
RdRp-RT-F	GTGTCCGGAGAGGTTAAGGATG
RdRp-RT-R	CTTGAATTGATCAACGGTGAACA
EcIRF3-RT-F	GACAACAAGAACGACCCTGCTAA
EcIRF3-RT-R	GGGAGTCCGCTTGAAGATAGACA
EcIRF7-RT-F	CAACACCGGATACAACCAAG
EcIRF7-RT-R	GTTCTCAACTGCTACATAGGG
EcISG15-RT-F	CCTATGACATCAAAGCTGACGAGAC
EcISG15-RT-R	GTGCTGTTGGCAGTGACGTTGTAGT
EcMXI-RT-F	CGAAAGTACCGTGGACGAGAA
EcMXI-RT-R	TGTTTGATCTGCTCCTTGACCAT
EcIL-1β-RT-F	AACCTCATCATCGCCACACA
EcIL-1β-RT-R	AGTTGCCTCACAACCGAACAC
EcIL-6-RT-F	GGTTGGTCCAAGGTGTGCTTA
EcIL-6-RT-R	CTGGGATTGTCGAGGTCCTT
EcIL-8-RT-F	GCCGTCAGTGAAGGGAGTCTAG
EcIL-8-RT-R	ATCGCAGTGGGAGTTTGCA
EcTNFα-RT-F	GTGTCCTGCTGTTTGCTTGGTA
EcTNFα-RT-R	CAGTGTCCGACTTGATTAGTGCTT

2.4. Plasmid construction

To clarify the subcellular localization and potential function of EcIFIT1 *in vitro*, the ORF of EcIFIT1 was subcloned into pEGFP-C1 or pcDNA3.1-3 × HA using the primers in Table 1 and the recombinant plasmids (pEGFP-EcIFIT1, pcDNA3.1-EcIFIT1) were both verified by DNA sequencing.

2.5. Cellular localization of EcIFIT1

To analyze the subcellular localization of EcIFIT1, GS cells cultured in 6-well plates with microscopic coverslips (25 × 25 mm) were transfected with the plasmids pEGFP-C1 or pEGFP-EcIFIT1 using the Lipofectamine 2000 reagent (Invitrogen) for 36 h and fixed with 4% paraformaldehyde (PFA) for 1 h at room temperature. Then cells were stained with 4, 6-diamidino-2-phenylindole (DAPI) for 5 min and observed under fluorescence microscopy [5,40].

2.6. Virus infection assay

To determine the roles of EcIFIT1 on SGIV and RGNNV replication, GS cells were transfected with pcDNA3.1-EcIFIT1 or pcDNA3.1-3 × HA

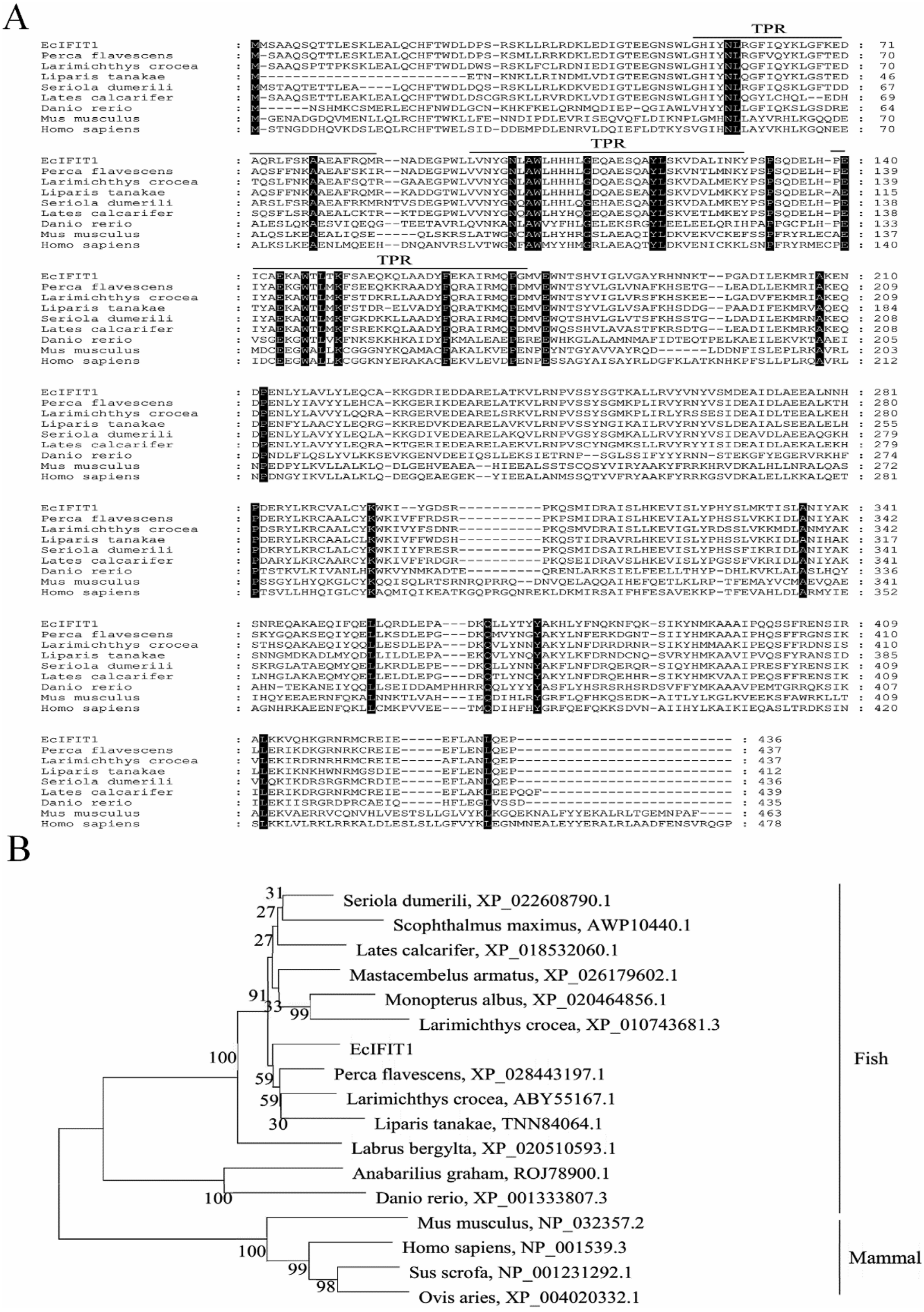

Fig. 1. Conserved domains and phylogenetic analysis of IFIT1 proteins. (A) Amino acid alignment of IFIT1s from different species. Accession numbers were listed as follows: *Perca flavescens*, XP_028443197.1; *Larimichthys crocea*, ABY55167.1; *Liparis tanakae*, TNN84064.1; *Seriola dumerili*, XP_022608790.1; *Lates calcarifer*, XP_018532060.1; *Danio rerio*, XP_001333807.3; *Mus musculus*, NP_032357.2; *Homo sapiens*, NP_001539.3. (B) Phylogenetic analysis of IFIT1s. A neighbor-joining tree was constructed based on the protein sequences of IFIT1-like genes from different species using MEGA 6.0 software. Numbers at the nodes denote the bootstrap values of 1000 replicates. Scale represents the numbers of substitutions per 1000 bases.

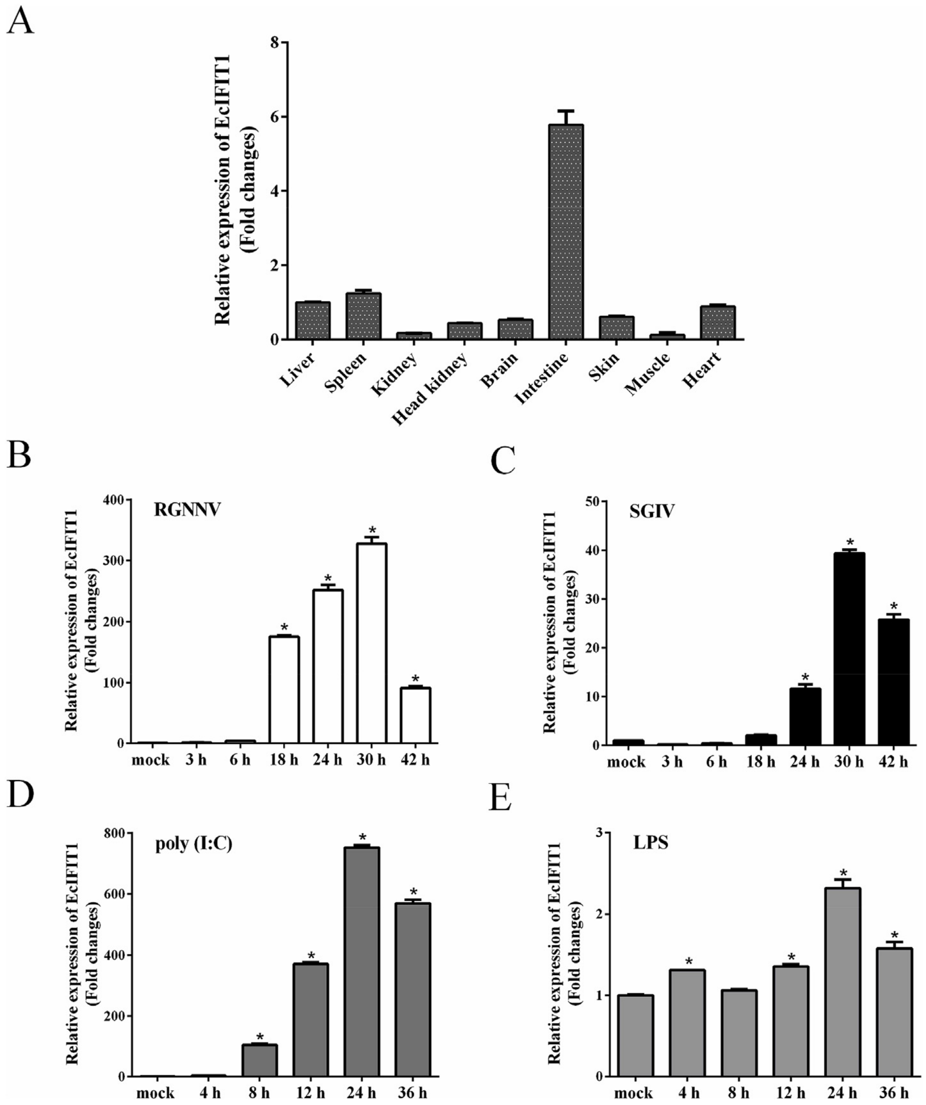

Fig. 2. The expression profiles of EcIFIT1 *in vitro*. (A) The expression level of EcIFIT1 in different tissues from healthy groupers. GS cells were infected with RGNNV (B) or SGIV (C) at MOI 2, transfected with 200 ng poly (I:C) (D) or pre-treated with 4 μg/mL LPS (E), and then collected at the indicated time points to detect the expression levels of EcIFIT1 by qPCR (n = 3, means ± SD). $^{*}P < 0.05$.

for 24 h, and infected with SGIV or RGNNV at MOI 2. Then cytopathic effect (CPE) were observed and photographed using a phase contrast microscope, besides, the mock- and virus-infected cells were harvested at 12 and 24 h.p.i. for RNA extraction and qPCR analysis.

Meanwhile, the whole-cell lysates of SGIV infected EcIFIT1-or control-overexpressing cells were collected at 24 h.p.i and the virus titers were examined as describe previously [40]. Briefly, GS cells were seeded in 96-well plates for 18–24 h, and then infected with serial 10-fold dilutions of SGIV samples in eight replicates. After 96–144 h.p.i., the data was calculated by the 50% tissue culture infective dose ($TCID_{50}$) using the Reed–Muench method.

2.7. RNA extraction and quantitative PCR

Total RNA was extracted using the SV Total RNA Isolation Kit (Promega), and reversed by ReverTra Ace qPCR RT Kit (TOYOBO). qPCR was carried out in Applied biosystems QuantStudio 5 Real Time Detection System (Thermofisher, USA) using the primers listed in Table 1. The cycling condition of each assay in triplicate was as follows: 95 °C for 1 min for activation, followed by 40 cycles at 95 °C for 15 s, 60 °C for 15 s and 72 °C for 45 s. The data was calculated with the $2^{-\Delta\Delta CT}$ method with β-actin as an internal reference and represented as mean ± SD.

2.8. Western blot assay

GS cells transfected with pcDNA3.1-EcIFIT1 or pcDNA3.1-3 × HA for 36 h were collected and lysed in RIPA buffer (Thermofisher). The total cell proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Millipore) for 60 min. The separated proteins were reacted with the indicated primary antibodies: anti-HA (1:1000 dilution) and *anti*-β-tublin (1:1000 dilution) for 2 h, and incubated with horseradish peroxidase (HRP)-conjugated sheep-mouse IgG or sheep-rabbit IgG at a dilution of 1:5000 for 2 h. Immunoblots were visualized using an enhanced HRP-DAB Substrate Chromogenic Kit (Tiangen) according to the manufacturer's protocol.

2.9. Flow cytometry analysis

To investigate the effects of EcIFIT1 on cell cycle progression, GS cells overexpressing pcDNA3.1-EcIFIT1 or pcDNA3.1-3 × HA were collected at 36 h post-transfection and fixed in 70% pre-cooled ethanol overnight at −30 °C. Cells were washed with PBS and centrifuged at

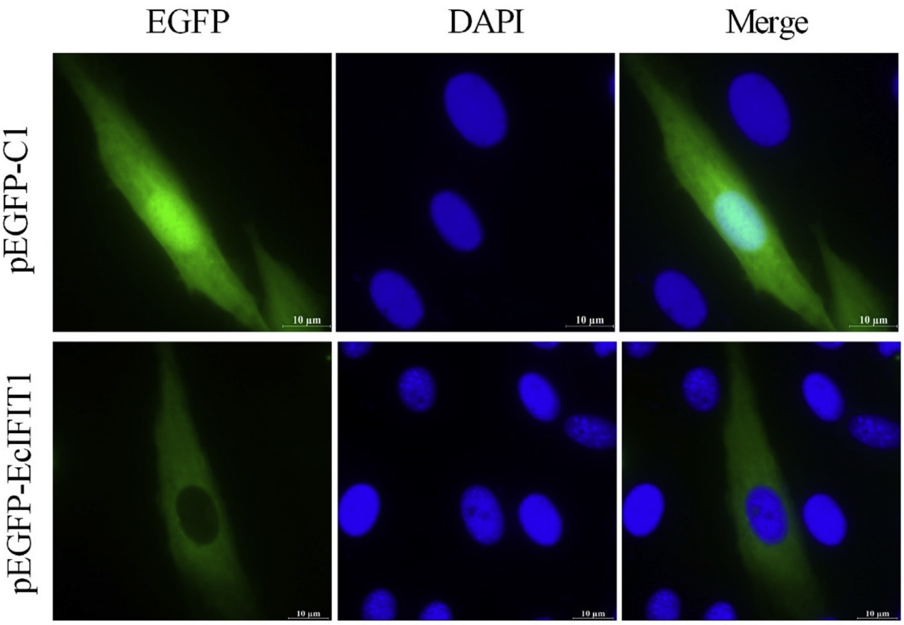

Fig. 3. Subcellular localization of EcIFIT1 in grouper cells. GS cells were transfected with pEGFP-C1 or pEGFP-EcIFIT1 for 36 h, and stained with DAPI. Samples were observed under fluorescence microscopy.

400 g for 10 min, and then incubated with PBS containing 50 μg/mL propidium iodide (PI) and 100 μg/mL RNaseA at room temperature for 30 min. The PI fluorescence was measured with a Beckman Coulter flow cytometer (Brea, USA), and 10,000 cells were selected for analysis in each sample. Each assay was carried out in triplicate. The data were analyzed using ModFit LT 4.1 software and represented as mean ± SD [43].

2.10. Statistical analysis

Statistics were performed using SPSS version 20 by one-way ANOVA. Differences were considered statistically significant when *p*-value was less than 0.05 ($*p < 0.05$).

3. Results

3.1. Sequence characterization of EcIFIT1

According to the EST sequences from grouper spleen transcriptome, the full length cDNA of EcIFIT1 was identified to be 1839 bp, consisting of a 5′-untranslated region (UTR) of 83 bp, a predicted ORF of 1311 bp, and a 3′-UTR of 445 bp with a polyadenylation signal sequence (AATAAA) and a poly(A) tail (GenBank accession no: MN150711) (Fig. S1). Sequence analysis showed that EcIFIT1 encoding a 436-amino-acid polypeptide shared 77.8% identity to IFIT1 homolog of yellow perch (*Perca flavescens*), but only 22.8% to humans (*homo sapiens*). Besides, EcIFIT1 contained three tetratricopeptide repeat (TPR) motifs formed by residues 53 to 86, 96 to 129, and 139 to 174 (Fig. 1A). Phylogenetic analysis indicated that EcIFIT1 showed the closest relationship to that of *P. flavescens*, *L. crocea*, and *Liparis tanakae*, and all the IFIT1s from fish were clustered into one group which was separated from mammals (Fig. 1B).

3.2. Expression patterns of EcIFIT1

To investigate the expression profiles of EcIFIT1, we firstly examined the transcription level of EcIFIT1 in various tissues from healthy grouper by qPCR. As shown in Fig. 2A, the expression of EcIFIT1 were detected in all the analyzed tissues, with the highest expression in the intestine, spleen, liver, and heart, followed by skin, brain, head kidney, kidney and muscle. Subsequently, we determined the expression changes of EcIFIT1 during different virus (RGNNV and SGIV) infection and PAMPs (poly (I:C) and LPS) treatment. As demonstrated in Fig. 2B, compared with mock-infected cells, EcIFIT1 expression was induced by RGNNV infection which increased from 18 h.p.i and reached a peak at up to 327-fold higher than of the mock-infected control cells at 30 h.p.i. During SGIV infection, the transcription levels of EcIFIT1 was increased up to 39-fold higher than the level attained in the mock-infected cells at 30 h.p.i. (Fig. 2C). Moreover, EcIFIT1 was induced significantly by poly (I:C) and LPS (Fig. 2D and E). Taken together, it was proposed that EcIFIT1 might play an important role in response to fish virus infection and PAMPs.

3.3. EcIFIT1 encoded a cytoplasmic protein

To explore the subcellular localization of EcIFIT1 *in vitro*, GS cells were transfected with pEGFP-EcIFIT1 or pEGFP-C1 for 36 h, and stained with DAPI. As shown in Fig. 3, in pEGFP-EcIFIT1 transfected cells, the green fluorescence was distributed in the cytoplasm. Differently, the green fluorescence in pEGFP-C1 transfected cells was observed throughout the cytoplasm and nucleus. Thus, it was suggested that EcIFIT1 encoded a cytoplasmic protein.

3.4. Overexpression of EcIFIT1 inhibited SGIV and RGNNV replication in vitro

To assess the effects of EcIFIT1 on the replication of SGIV and RGNNV, we determined the severity of CPE, the transcription level of virus genes by qPCR and the virus titer by $TCID_{50}$. Firstly, the expression level of EcIFIT1 was examined by qPCR and western bolt to ensure its successful overexpression in transfected cells. As shown in Fig. 4A, compared with the control vector cells, the transcription level of EcIFIT1 was significantly increased up to 247-fold, and the recombinant plasmid successfully expressed IFIT1 protein. EcIFIT1 overexpression obviously wakened the severity of CPE induced by SGIV and RGNNV infection at 24 h.p.i. (Fig. 4B). Consistently, in EcIFIT1 overexpressing cells, the transcription level of SGIV major capsid protein (MCP), VP19, and RGNNV the capsid protein (CP), RNA-dependent RNA-polymerase

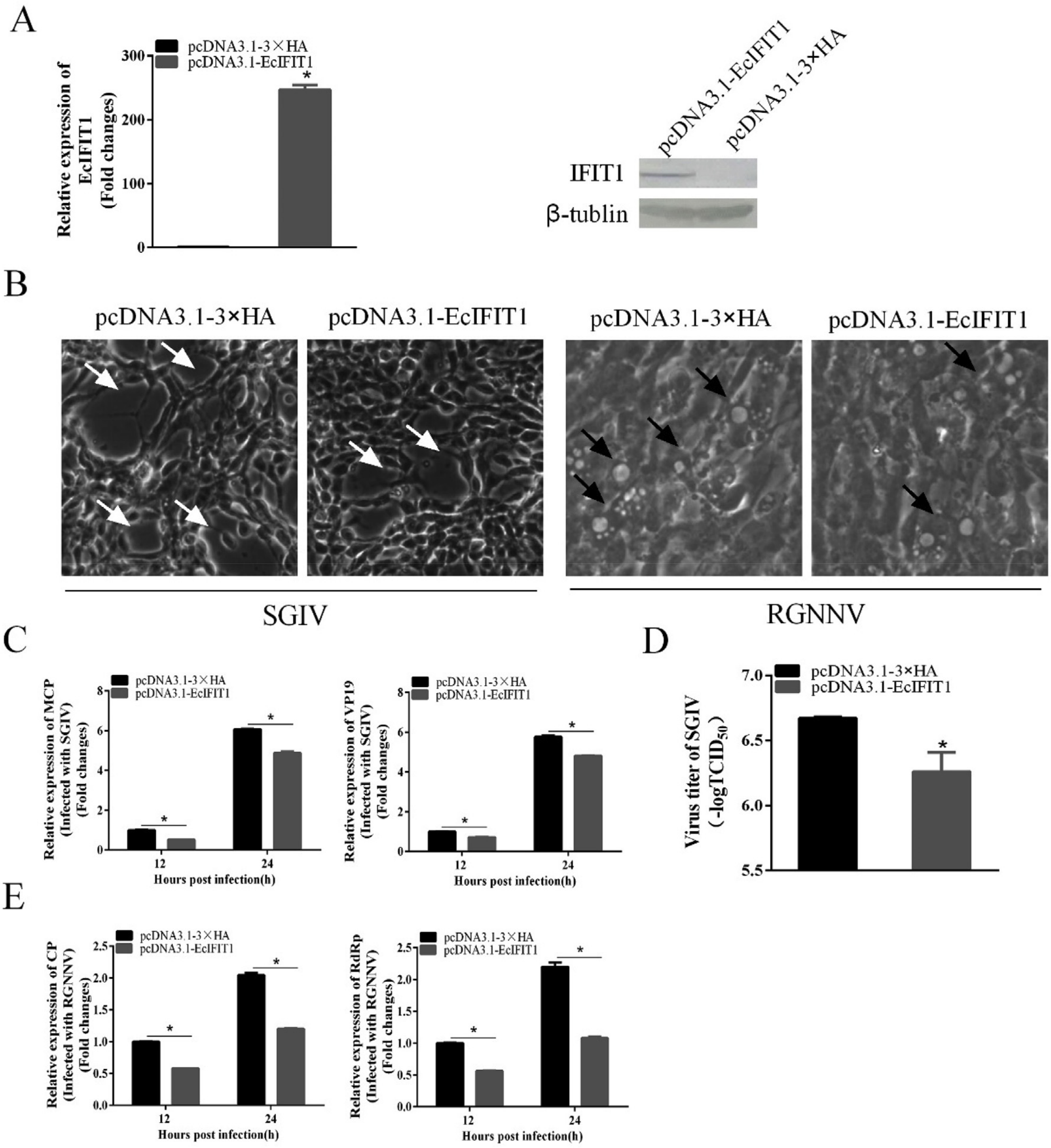

Fig. 4. EcIFIT1 overexpression significantly inhibited SGIV and RGNNV replication. (A) The transcription and protein level of EcIFIT1 in EcIFIT1-overexpressing cells. After transfection with pcDNA3.1-3 × HA or pcDNA3.1-EcIFIT1, GS cells were harvested at 36 h post-transfection to evaluate the expression level of EcIFIT1 by qPCR and western blot assay. (B) The severity of CPE induced by RGNNV and SGIV was weakened by EcIFIT1 overexpression. After transfection, GS cells were infected with SGIV or RGNNV, and the cellular morphology was observed using microscopy. The white arrows and the black arrows indicated the severity of CPE induced by SGIV infection and the vacuoles induced by RGNNV infection, respectively. (C,E) Overexpression of EcIFIT1 decreased the viral gene transcription of SGIV and RGNNV. EcIFIT1 overexpressing cells were infected with SGIV (C) or RGNNV (E), and collected at 12 and 24 h.p.i. to determine the transcription level of viral gene by qPCR. (D) EcIFIT1 overexpression reduced SGIV production. EcIFIT1 overexpressing cells infected with SGIV at 24 h.p.i were harvested to examine the virus titer by $TCID_{50}$. (n = 3, means ± SD). $^{*}P < 0.05$.

(RdRp), were all significantly decreased compared to the control cells (Fig. 4C,E). Besides, the virus titer assay indicated that EcIFIT1 significantly reduced SGIV production (Fig. 4D). Together, the results indicated that EcIFIT1 exerted antiviral effect against fish DNA and RNA virus infection.

3.5. EcIFIT1 overexpression differently regulated IFN immune and pro-inflammatory response

To demonstrate the potential antiviral mechanism of EcIFIT1, we determined the mRNA expression of host IFN immune related molecules and proinflammatory cytokines in EcIFIT1 overexpressing cells by qPCR. GS cells were transfected with pcDNA3.1-EcIFIT1 or pcDNA3.1-3 × HA for 36 h, and collected for RNA extraction and qPCR analysis. As shown in Fig. 5A, EcIFIT1 overexpression significantly increased the transcription level of IFN signaling molecules, including IRF3, IRF7, ISG15 and myxovirus resistance gene (MX) I. In addition, the expression of proinflammatory cytokines were differently regulated by EcIFIT1 overexpression. In detail, compared with the control vector cells, the expression of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α were significantly increased, while that of IL-8 was decreased in EcIFIT1 overexpressing cells (Fig. 5B). Therefore, the overexpression of EcIFIT1 *in vitro* differently regulated the IFN and pro-inflammatory response.

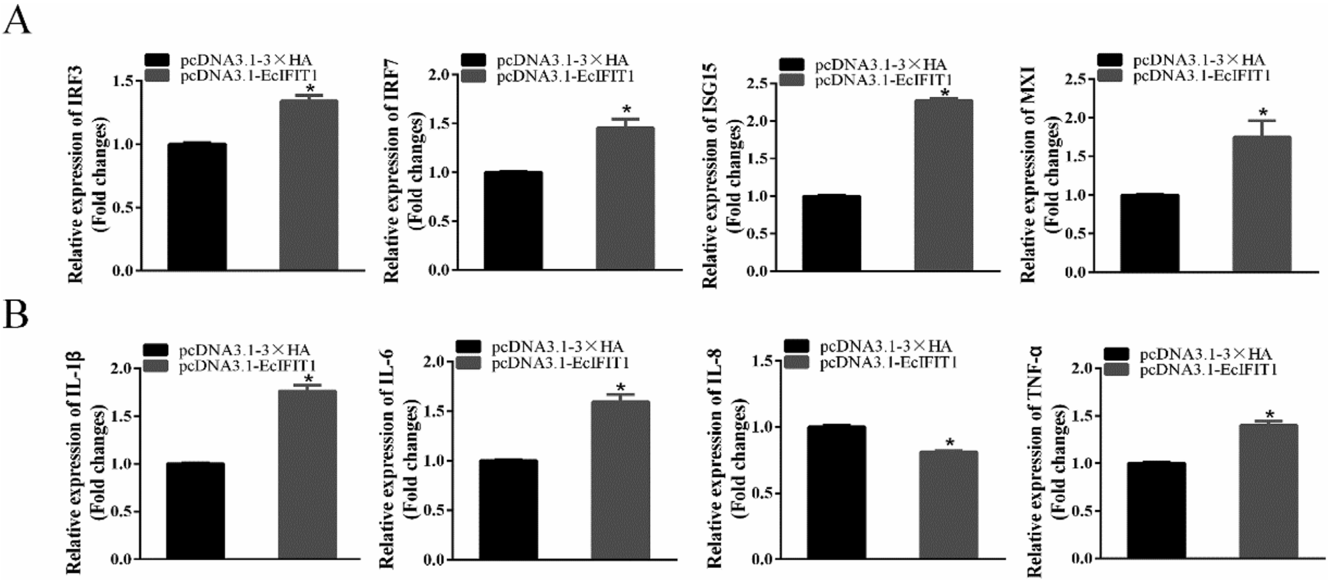

Fig. 5. Overexpression of EcIFIT1 regulated the expression level of IFN related signaling molecules and proinflammatory cytokines. (A) EcIFIT1 overexpression positively regulated the IFN immune response. The relative expression level of IFN signaling molecules including IRF3, IRF7, ISG15 and MXI in EcIFIT1 overexpressing cells were detected by qPCR. (B) Proinflammatory cytokines including IL-1β, IL-6, IL-8 and TNF-α were differently regulated in EcIFIT1 overexpressing cells. (n = 3, means ± SD). *$P < 0.05$.

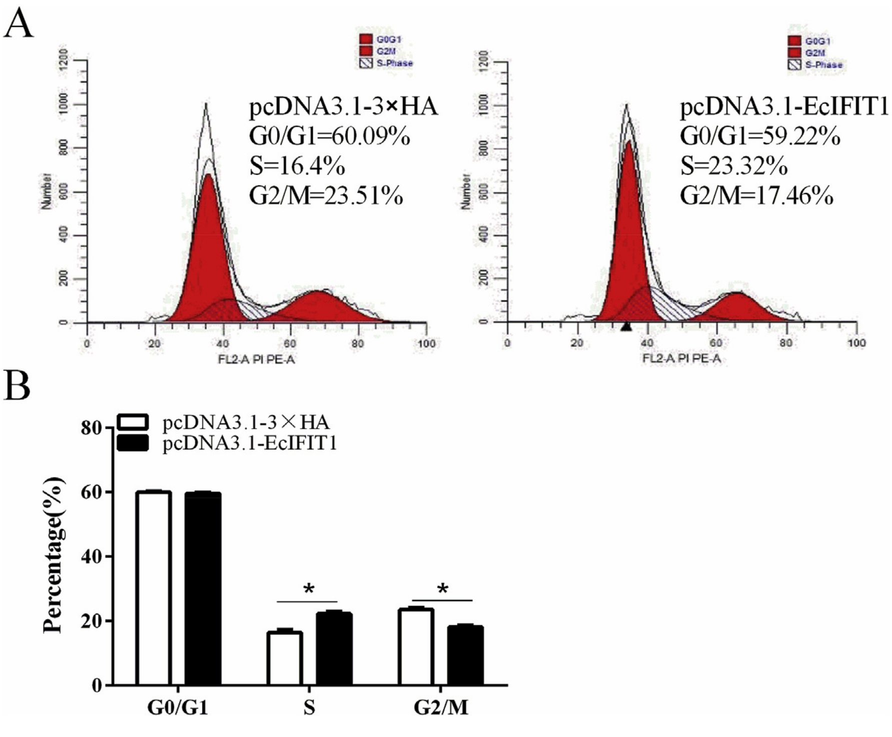

Fig. 6. The effects of EcIFIT1 on cell cycle progression. (A) The cell cycle progressions of control- and EcIFIT1-overexpressing cells. GS cells were transfected with pcDNA3.1-3 × HA or pcDNA3.1-EcIFIT1, and collected at 36 h post-transfection for flow cytometry analysis. (B) Quantitative analysis of the percentages of cells in different cell phase. (n = 3, means ± SD). *$P < 0.05$.

3.6. Overexpression of EcIFIT1 affected the cell cycle progression

It has been reported that IFITs play a crucial role in regulating cell cycle progression [15,44]. However, whether EcIFIT1 exerted similar roles on cell cycle remained uncertain. To explore the role of EcIFIT1 on cell cycle progression, GS cells were transfected with pcDNA3.1-EcIFIT1 or pcDNA3.1-3 × HA for 36 h, and harvested for flow cytometry analysis. As shown in Fig. 6A, the percentages of the S phase cells in pcDNA3.1-3 × HA- or pcDNA3.1-EcIFIT1-overexpressing cells were 16.4% and 23.32%, respectively. Compared to the mock, overexpression of EcIFIT1 obviously inhibited the S/G2 transition and arrested cells in S phase (Fig. 6B). Thus, the results demonstrated that EcIFIT1 could affect cell cycle progression from the S to the G2 phase and arrest cells in the S phase.

4. Discussion

Increasing evidences have demonstrated that IFIT1 inhibits virus replication in mammalian cells through different mechanisms [23,25,45]. Moreover, the antiviral activity of IFIT1 was demonstrated during both DNA and RNA virus infection. However, the roles of IFIT1 in lower vertebrates remained largely unknown. Here, we cloned an IFIT1 homolog from grouper and investigated the effects of EcIFIT1 on SGIV and RGNNV infection.

Based on the EST data from grouper spleen transcriptome [42], we obtained the complete cDNA of EcIFIT1 by the RACE method. Sequence analysis showed that EcIFIT1 encoded a 436-amino-acid polypeptide, which shared 77.8% identity to that from *P. flavescens*. Consistent with the structure of other teleosts IFIT1s [29,32], EcIFIT1 contained three TPR motifs, which was composed of 34 amino acids. Besides, the results indicated that EcIFIT1 expression was increased significantly during SGIV and RGNNV infection, which was similar to the studies in which IFIT1 was induced by viral hemorrhagic septicemia virus (VHSV) [32], LCMV [24], megalocytivirus [29], grass carp hemorrhage virus (GCHV) [30], and avian influenza virus H9N2 [46]. In addition, studies have been reported that IFIT1 could be induced by PAMPs, such as poly (I:C) [32,47] and LPS [48], which were consistent with the results of the present study, suggesting that EcIFIT1 plays a crucial role in the innate immune response against fish virus infection.

As mentioned above, previous studies have demonstrated that IFIT1 have broad antiviral activity against some viruses. For example, overexpression of IFIT1 significantly decreased the replication of megalocytivirus, whereas knockdown of IFIT1 by siRNA, increased viral replication in tongue sole [29]. Similarly, HCV replication was inhibited by the ectopic overexpression of IFIT1, and enhanced by the knockdown of IFIT1 [20]. Besides, knockdown of IFN-induced proteins with IFIT1 led to markedly increase hepatitis B virus (HBV) replication [49]. In our study, the severity of CPE induced by SGIV and RGNNV infection were significantly weakened by EcIFIT1 overexpression. Moreover, the ectopic expression of EcIFIT1 not only inhibited the transcription of viral genes, including SGIV MCP, VP19 and RGNNV RdRp, CP, but also decreased the titer of SGIV, indicating that EcIFIT1 also exerted antiviral functions against DNA and RNA virus infection.

It has been demonstrated that IFIT1 was involved in IFN immune response and physiological inflammation in mammals [47,50,51]. For example, IFIT1 knockdown decreased the protein and mRNA levels of retinoic acid inducible gene-I (RIG-I) and MDA5 [47]. Moreover, IFIT1 upregulated the expression of a chemokine (C-X-C motif) ligand (CXCL) 10 induced by LPS [48]. Consistently, in the present study, we found that EcIFIT1 overexpression significantly increased the expression of several IFN related cytokines, including IRF3, IRF7, ISG15 and MXI, as well as that of inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, while that of IL-8 was significantly decreased. Taken together, it was speculated that the positive regulation of IFN immune and proinflammatory response by EcIFIT1 might be crucial for its antiviral action against SGIV and RGNNV infection.

In addition, it has been reported that several IFITs proteins could regulate cell cycle progression. Knockdown of IFIT2 significantly promoted cell growth, triggered migration and invasion [52]. Besides, IFIT3 expression upregulated p21 and p27, which were negative regulators of the cell cycle progression from G1 into S phase [44]. Our results showed that EcIFIT1 overexpression altered the transition of S/G2, and arrested cells in S phase, suggesting that the EcIFIT1 was able to regulate cell cycle progression. Given that cell cycle was also crucial for SGIV and RGNNV infection [10,43], we speculated that the effect of EcIFIT1 on cell cycle might affect virus replication. While the potential molecular mechanism needed further investigation.

In conclusion, a novel IFIT1 homolog from orange spotted grouper (EcIFIT1) was cloned and characterized in this study. EcIFIT1 encoded a cytoplasmic protein in grouper cells, and could be induced by RGNNV or SGIV infection. *In vitro*, overexpression of EcIFIT1 significantly suppressed the replication of SGIV and RGNNV via positively regulating the host IFN immune response and affecting the cell cycle. Our results will contribute greatly to understanding the roles of fish IFIT1 in response to viruses.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.08.075.

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