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Grouper DDX41 exerts antiviral activity against fish iridovirus and nodavirus infection

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ABSTRACT

DEAD (Asp-Glu-Ala-Asp)-box polypeptide 41 (DDX41) is a member of the DEXDc family of helicases, that has recently been identified to be a crucial intracellular DNA sensor that triggers multiple signaling molecules to activate the type I interferon response. However, the precise function of DDX41 in fish during a viral infection remains unknown. In the present study, the DDX41 homolog from orange spotted grouper, *Epinephelus coioides* (EcDDX41), was cloned and its potential role in the immune response to a fish viral infection were investigated. EcDDX41 encodes a putative protein of 614 amino acid residues that contained two conserved domains: 1) DEADc domain; and 2) HELICc domain. The sequence analysis indicated that EcDDX41 shared 99%, 94%, and 86% identity with Asian seabass (*Lates calcarifer*), zebrafish (*Danio rerio*), and humans (*Homo sapiens*), respectively. EcDDX41 mRNA was present in all of the detected tissues, with the highest level of expression in the gills. The level of EcDDX41 expression was up-regulated following infection with Singapore grouper iridovirus (SGIV) or red-spotted grouper nervous necrosis virus (RGNNV) in grouper spleen (GS) cell cultures, suggesting that EcDDX41 may be involved in fish virus infection. Furthermore, EcDDX41 overexpression in GS cells significantly inhibited SGIV and RGNNV replication. EcDDX41 overexpression significantly increased the expression of antiviral and inflammatory cytokine genes, including interferon regulatory factor genes (e.g., IRF1, IRF2, IRF3, and IRF7), interferon induced genes (e.g., ISG15, ISG56, IFP35, Viperin, and MXI), and pro-inflammatory cytokine genes (e.g., TNFα, IL-1β, and IL-8). Moreover, EcDDX41 positively regulated the mitochondrial antiviral-signaling protein (MAVS) and TANK-binding kinase 1 (TBK1)-induced interferon immune response, but did mediate IRF3 activation (MITA) to evoke an interferon immune response in unstimulated cells. Together, our results provide novel insight into the role of fish DDX41 in the antiviral innate immune response.

1. Introduction

Increased evidence has demonstrated that human DExD/H RNA helicases (DDX) play a crucial role in multiple cellular processes, including cell survival, cell death, tumorigenesis, and innate immunity [1–3]. Among these helicases, DDX41 was first identified as an intracellular DNA sensor in myeloid dendritic cells, and the knockdown of DDX41 expression blocked the activation of the protein kinase, TANK-binding kinase 1 (TBK1), and the transcription factors, nuclear factor kappa beta (NF-κB) and interferon regulatory factor-3 (IRF3) [4]. In addition, DDX41 was also found to be required for the antiretroviral innate immune response to murine leukemia virus (MLV) dendritic cells [5,6]. In addition, the role of DDX41 in innate immunity was also found to be involved in response to other diseases (e.g., myelodysplastic syndrome or acute myeloid leukemia [MDS/AML] syndrome) [7]. In lower vertebrates, flounder DDX41 was found to induce antiviral and inflammatory cytokine gene expression in response to treatment with cytoplasmic C-di-GMP [8]. Moreover, zebrafish DDX41-mediated signaling pathways have been demonstrated to play an important role in innate antibacterial immunity [9]. However, the actions and potential mechanism of fish DDX41 against viral infection still remain poorly understood.

Groupers are important aquaculture species in China and other Southeast Asian countries due to its important economic value. However, an increasing number of viral infectious diseases caused by Singapore grouper iridovirus (SGIV) or red-spotted grouper nervous

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Table 1
Primers used in this study.

Name	Sequence (5′–3′)
EcDDX41-N3/C1–F	TCCAGGTACCATGGAGACCGAAAATCGACCA
EcDDX41-N3/C1-R	AATGGATCCGAAGTCCATTGAGCTGTGAGCC
EcDDX41-Flag-F	TCAAGGTACCAATGGAGACCGAAAATCGACCA
EcDDX41-Flag-R	TGTAGGATCCGAAGTCCATTGAGCTGTGAGCC
EcDDX41-RT-F	CGATTATGTGCCGTATGTTCCTG
EcDDX41-RT-R	TCCTTGGCTGACTCCTTACGAG
Actin-RT-F	TACGAGCTGCCTGACGGACA
Actin-RT-R	GGCTGTGATCTCCTTCTGCA
RGNNV-CP-RT-F	CAACTGACAACGATCACACCTTC
RGNNV-CP-RT-R	CAATCGAACACTCCAGCGACA
RdRp-RT-F	GTGTCCGGAGAGGTTAAGGATG
RdRp-RT-R	CTTGAATTGATCAACGGTGAACA
SGIV-MCP-RT-F	GCACGCTTCTCTCACCTTCA
SGIV-MCP-RT-R	AACGGCAACGGGAGCACTA
SGIV-VP19-RT-F	TCCAAGGGAGAAACTGTAAG
SGIV-VP19-RT-R	GGGGTAAGCGTGAAGACT
SGIV-LITAF-RT-F	GATGCTGCCGTGTGAACTG
SGIV-LITAF-RT-R	GCACATCCTTGGTGGTGTTG
SGIV-ICP18-RT-F	ATCGGATCTACGTGGTTGG
SGIV-ICP18-RT-R	CCGTCGTCGGTGTCTATTC
EcIRF3-RT-F	GACAACAAGAACGACCCTGCTAA
EcIRF3-RT-R	GGGAGTCCGCTTGAAGATAGACA
EcIRF7-RT-F	CAACACCGGATACAACCAAG
EcIRF7-RT-R	GTTCTCAACTGCTACATAGGGC
EcISG15-RT-F	CCTATGACATCAAAGCTGACGAGAC
EcISG15-RT-R	GTGCTGTTGGCAGTGACGTTGTAGT
EcTNFa-RT-F	GTGTCCTGCTGTTTGCTTGGTA
EcTNFa-RT-R	CAGTGTCCGACTTGATTAGTGCTT
EcIL-1β-RT-PF	AACCTCATCATCGCCACACA
EcIL-1β-RT-PR	AGTTGCCTCACAACCGAACAC
EcIL8-RT-F	GCCGTCAGTGAAGGGAGTCTAG
EcIL8-RT-R	ATCGCAGTGGGAGTTTGCA
EcIFP35-RT-F	TTCAGATGAGGAGTTCTCTCTTGTG
EcIFP35-RT-R	TCATATCGGTGCTCGTCTACTTTCA
EcMXI-RT-F	TGTTTGATCTGCTCCTTGACCAT
EcMXI-RT-R	GGATGAAGACATGACGGAGAACA

necrosis virus (RGNNV) have frequently occurred and accompanied by the large-scale seed production of groupers in recent years [10,11]. To obtain a better understanding of the molecular pathogenesis mechanisms of SGIV and RGNNV infections, several interferon-related cytokines or effectors have been identified to exert crucial roles in viral replication in groupers [12–14]. For example, interferon stimulated gene-15 (ISG15) and IRF3 exhibited antiviral activity in response to RGNNV infection, but not to SGIV. In addition, grouper melanoma differentiation-associated gene 5 (MDA5) and TBK1 significantly inhibited both RGNNV and SGIV replication *in vitro*. Moreover, grouper tripartite motif 62 (TRIM62) and tripartite motif 16 like (TRIM16L) negatively regulated the antiviral immune response and enhanced viral replication [14,15]. We recently demonstrated that grouper DDX3X displayed critical antiviral activity against RGNNV, but not SGIV. EcDDX3X overexpression evoked the activation of an IFN immune response via both IRF3 and IRF7 [16]. However, whether other DDX family members are involved in the response to SGIV or RGNNV infection still remained uncertain. In the present study, we sought to provide novel insight into the role of fish DDX41 in the antiviral innate immune response. A DDX41 homolog from grouper, *Epinephelus coioides*, was cloned and characterized. The subcellular localization of grouper DDX41 (EcDDX41) was investigated in transfected cells. The effects of EcDDX41 on viral replication and the host immune response were also evaluated.

2. Material and methods

2.1. Virus and cells

Grouper spleen (GS) cells were cultured at 28 °C in Leibovitz's L15 medium containing 10% fetal bovine serum (FBS, Gibco) [17]. The viruses used in this study included SGIV and RGNNV, which were prepared as described previously [17,18].

2.2. Cloning and sequence analysis of EcDDX41

Based on the assembly of EST sequences from the grouper spleen transcriptome [18], the full-length cDNA of EcDDX41 was amplified via PCR and verified by DNA sequencing. The deduced amino acid (AA) sequences of EcDDX41 were subjected to BLAST program analysis in the NCBI database. Multiple sequence alignment of the DDX41 homologs was performed using ClustalX1.83 software and edited with the GeneDoc program. A phylogenetic tree was constructed using Mega 5.0 software [19].

2.3. Expression patterns of EcDDX41

To clarify the distribution pattern of EcDDX41 transcription, different tissues were collected from anesthetized healthy groupers (*E. coioides*) (50 g − 60 g) (n = 3), which were kept in a recirculating seawater system, including the head kidney, heart, liver, spleen, intestine, muscle, brain, skin, gill, stomach, and kidney [20]. The total RNA was extracted and the level of EcDDX41 expression was examined using quantitative real-time PCR (qPCR) as described below.

To analyze the expression profiles of EcDDX41 against fish virus infection, GS cells were infected with SGIV or RGNNV at a MOI of 1. Then, cells were collected at 4, 12, 24, and 48 h post-infection (hpi) for RNA extraction and further qPCR analysis.

2.4. RNA extraction and qPCR analysis

Total RNA from the grouper tissues or cells was extracted using a SV Total RNA Isolation System (Promega, USA) and reverse-transcribed using a ReverTra Ace qPCR RT Kit (Toyobo) as described previously [20]. qPCR was performed using a Roche 480 Real Time Detection System (Roche, German) as described previously [20]. Briefly, each assay was carried out under the following cycling conditions: 95 °C for 5 min for activation, followed by 45 cycles at 95 °C for 5 s, 60 °C for 10 s, and 72 °C for 15 s. The primers are listed in Table 1. All samples were performed in triplicate and the level of target gene expression was normalized to β-actin and calculated using the $2^{-\triangle\triangle CT}$ method. The data are represented as the mean ± SD (n = 3).

2.5. Plasmid construction

To elucidate the potential function of EcDDX41 during fish virus infection, EcDDX41 was cloned into pEGFP-C1 (Clontech, tagged with EGFP at its N-terminal), pEGFP-N3 (Clontech, tagged with EGFP at its C-terminal), and pcDNA3.1-Flag vectors. The corresponding recombinant plasmids, including pEGFP-EcDDX41, EcDDX41-pEGFP, and Flag-EcDDX41 were validated by DNA sequencing.

2.6. Cell transfection

GS cells were seeded into 24-well plates (80% confluent), and transfection was carried out using a transfection reagent (TA) Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Briefly, the expression plasmids (800 ng/well) were mixed with Lipofectamine 2000 (2 μL), added to the cells, and incubated for 6 h. After replacing the cultures with fresh medium, the cells were cultured at 25 °C for further study.

2.7. Fluorescence microscopy

To examine the subcellular localization of EcDDX41 in grouper cells, the plasmids, including pEGFP-C1, EcDDX41-pEGFP and pEGFP-

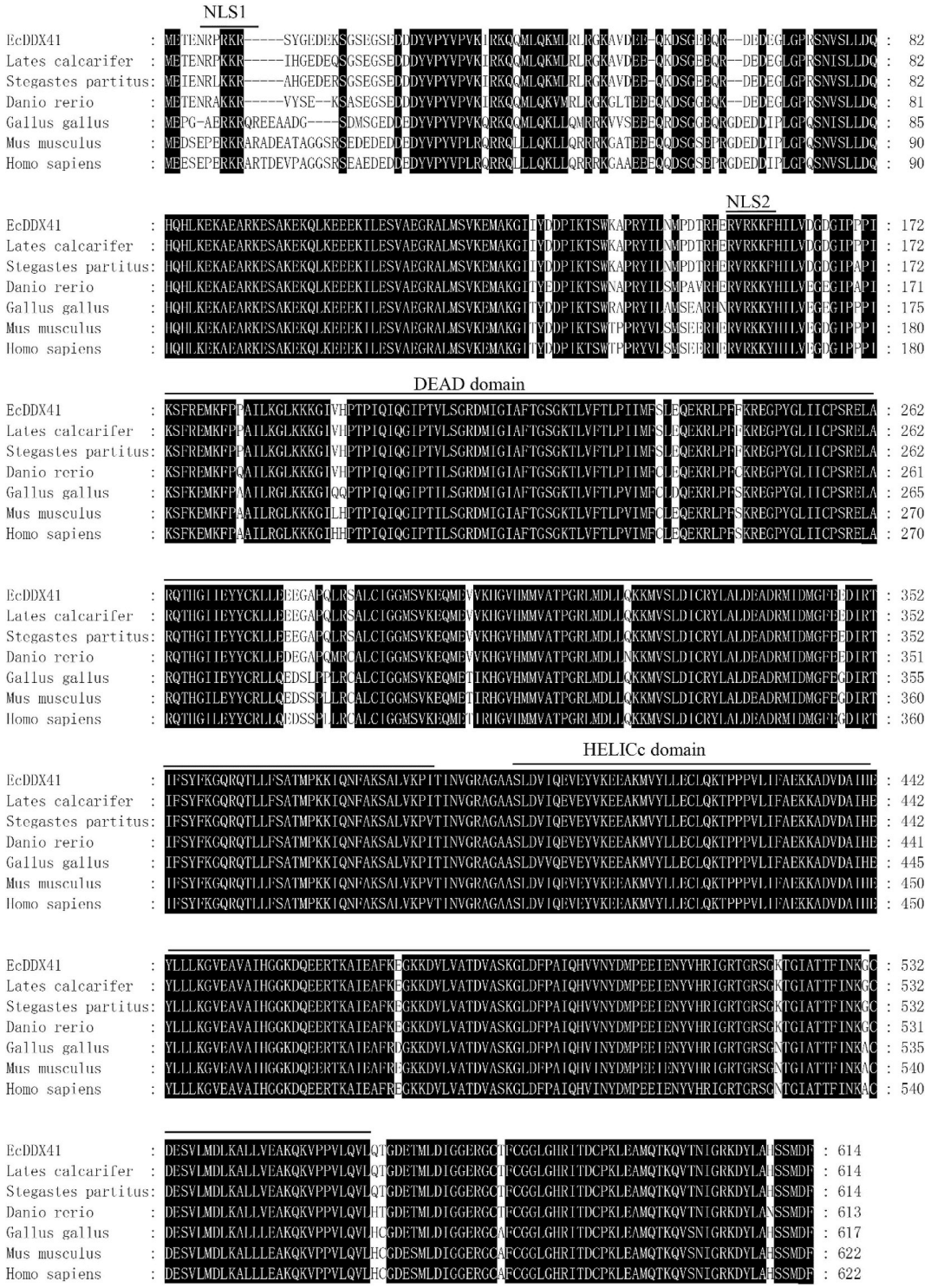

Fig. 1. Sequence alignment of the DDX41 homologs from different species. The conserved domains, including NLS1, NLS2, DEAD, and HELICc domain are underlined.

EcDDX41 plasmids were transfected into GS cells as described above. At 48 h post-transfection, the cells were fixed and stained with DAPI. The samples were observed using fluorescence microscopy (Zeiss).

To detect the endogenous DDX41 in grouper cells, immune fluorescence assay was performed as described previously [12]. In brief, mock or SGIV-infected GS cells were fixed, and then incubated with *anti*-DDX41 antibody. After washing with PBS, cells were incubated with FITC-conjugated secondary antibody, and then stained with DAPI. The fluorescence were observed under fluorescence microscopy. In addition, the protein synthesis of SGIV MCP and RGNNV CP in virus infected cells were also examined using immune fluorescence assay as described above.

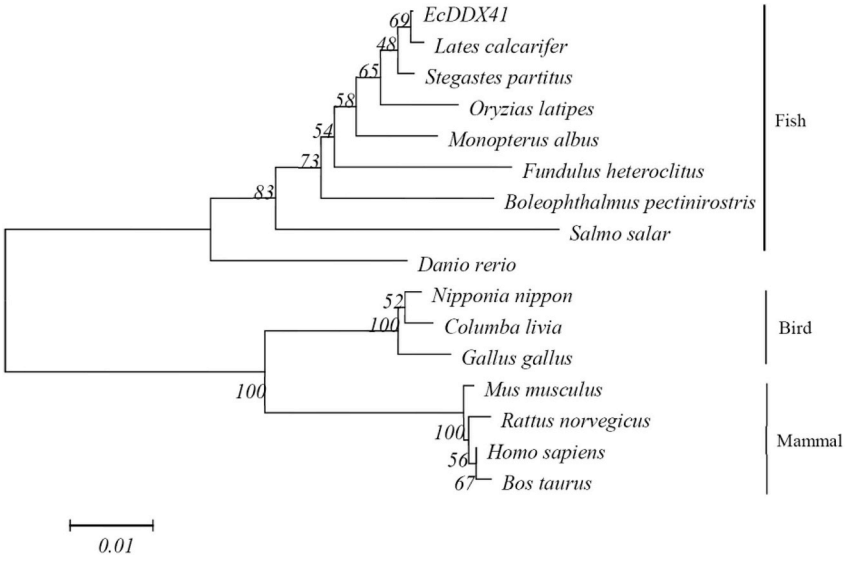

Fig. 2. Phylogenetic analysis of EcDDX41 and other DDX41 homologs. A phylogenetic tree was constructed using MEGA 5.0 with the neighbor-joining (NJ) method. The GenBank accession numbers are listed as follows: Barramundi (*Lates calcarifer*), XP_018531125; bicolor damselfish (*Stegastes partitus*), XP_008279565; medaka (*Oryzias latipes*), XP_004073424; rice eel (*Monopterus albus*), XP_020445571; mudskipper (*Boleophthalmus pectinirostris*), XP_020784282; mummichog (*Fundulus heteroclitus*), XP_012719536; Atlantic salmon (*Salmo salar*), NP_001133799; zebrafish (*Danio rerio*), XP_017209912; crested ibis (*Nipponia nippon*), KFR05092; rock pigeon (*Columba livia*), XP_013222816; red junglefowl (*Gallus gallus*), NP_001336637; house mouse (*Mus musculus*), NP_598820; brown rat (*Rattus norvegicus*), NP_001101516; human (*Homo sapiens*), NP_057306; and cattle (*Bos taurus*), NP_001076071.

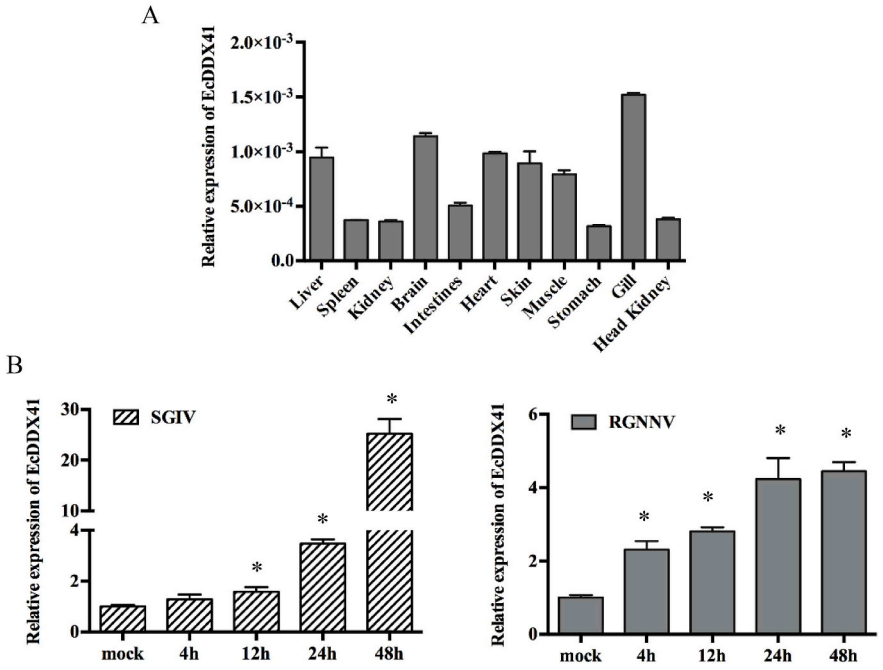

Fig. 3. The EcDDX41 expression profiles. (A) The tissue distribution patterns of EcDDX41 in healthy grouper. (B) The EcDDX41 expression profiles in grouper cells following an incubation with SGIV or RGNNV. $p < 0.05$ (*).

2.8. The effect of EcDDX41 on viral replication

To clarify the effect of EcDDX41 on fish virus replication, the level of viral core gene transcription was examined in EcDDX41-over-expressing cells. Briefly, GS cells were transfected with Flag-EcDDX41 or control plasmids, and subsequently incubated with SGIV or RGNNV at 24 h post-transfection. At the indicated time points (12 and 24 hpi), the virally-infected cells were collected, and the level of viral gene expression, including SGIV MCP (major capsid protein), ICP18, VP19, LITAF (LPS-induced TNF-α factor), RGNNV CP (coat protein), and RdRp (RNA-dependent RNA polymerase) genes were detected using qPCR as described above. Protein synthesis of the viral genes was detected using an immune fluorescence assay as described previously [14].

2.9. Evaluation of the EcDDX41-induced immune response in fish cells

To evaluate the effect of EcDDX41 on the immune response in fish cells, the level of interferon-related gene expression, including IRF3, IRF7, IRF1, IRF2, IFP35, and MXI and pro-inflammatory factors, including TNFα, IL-8, and IL-1β were also examined in EcDDX41-transfected cells. All the primers are listed in Table 1.

To further detect the regulatory roles of EcDDX41 on MAVS, mediator of IRF3 activation (MITA), or TBK1 evoked interferon response, EcDDX41 was co-transfected with MAVS, MITA or TBK1, and then the expression levels of interferon-related genes were evaluated using qPCR as described previously [15].

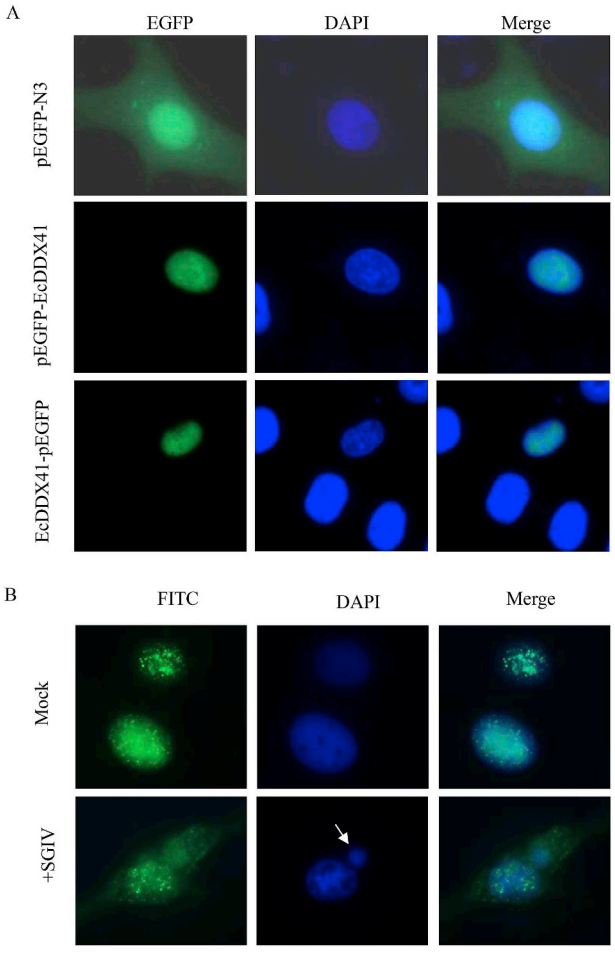

Fig. 4. Subcellular localization of EcDDX41 in grouper cells. (A) Localization of EcDDX41 in transfected cells. Recombinant plasmids, including pEGFP-EcDDX41, EcDDX41-pEGFP, and pEGFP-N3 (control vector) were transfected into grouper cells and subsequently stained with DAPI. (B) Localization of endogenous EcDDX41 in grouper cells. Mock- or SGIV-infected cells were fixed and incubated with an *anti*-DDX41 antibody. Fluorescence was observed using fluorescence microscopy. Arrows denote the virus assembly sites during SGIV infection. Magnification: × 100.

2.10. Statistic analysis

All statistical analyses were carried out with SPSS 15.0 software using a one-way analysis of variance (ANOVA). The level of significance was defined as $p < 0.05$ (*).

3. Results

3.1. EcDDX41 characterization

After the assembly of the EcDDX41 EST sequences from the grouper transcriptome, we validated the full-length cDNA of EcDDX41 using PCR amplification and DNA sequencing. The sequence analysis indicated that EcDDX41 encoded a putative protein of 614 AA residues which showed 99%, 94%, and 86% identity to Asian seabass (*Lates calcarifer*), zebrafish (*Danio rerio*), and humans (*Homo sapiens*), respectively. The AA alignment showed that EcDDX41 contained two conserved domains, including a DEADc and HELICc domain (Fig. 1). The AA-altering variations between fish and mammals were primarily present at the N terminal (approximately 70 AA), and two NLS motifs (NLS1 and NLS2) were predicted in EcDDX41. A phylogenetic analysis indicated that EcDDX41 exhibited the closest relationship to Asian seabass, followed by other fish, birds, and mammals (Fig. 2).

3.2. EcDDX41 expression patterns

The tissue distribution pattern of EcDDX41 transcription in grouper was investigated using qPCR. As shown in Fig. 3A, the expression of EcDDX41 was predominantly observed in the gill, followed by the brain, liver, heart, skin, muscle, intestines, spleen, kidney, head kidney, and stomach. The EcDDX41 expression profiles in grouper virus-infected cells were further analyzed. The results showed that the level of EcDDX41 expression was significantly up-regulated in response to both SGIV and RGNNV infection, suggesting that EcDDX41 might be involved in the immune response against fish virus infections (Fig. 3B).

3.3. EcDDX41 localized in the nucleus

To detect the subcellular localization of EcDDX41, we constructed the recombinant plasmids (pEGFP-EcDDX41 and EcDDX41-pEGFP) by tagging the green fluorescent protein to the N- or C-termini of EcDDX41. Using fluorescence microscopy, we found that green fluorescence was present only in the nucleus in both pEGFP-EcDDX41 and EcDDX41-pEGFP-transfected cells. In contrast, green fluorescence was observed throughout the cytoplasm and nucleus in the pEGFP-N3 or pEGFP-C1 (data not shown) transfected cells (Fig. 4A). In addition, the distribution of endogenous EcDDX41 was also detected using an *anti*-DDX41 antibody. The results showed that EcDDX41 was localized only in the nucleus. Moreover, EcDDX41 partially translocated into the cytoplasm during SGIV infection (Fig. 4B). Thus, our results demonstrate that EcDDX41 encodes a nucleus-localized protein in grouper cells.

3.4. EcDDX41 overexpression decreased virus replication

To clarify the potential role of EcDDX41 on fish virus replication, we evaluated the level of viral gene replication of SGIV and RGNNV in EcDDX41-overexpressing cells. The expression of EcDDX41 in EcDDX41-overexpressing cells was confirmed by an immune fluorescence assay (Supplemental Fig. 1). As shown in Fig. 5, the transcription of SGIV MCP, VP19, ICP-18, and LITAF was significantly inhibited in EcDDX41-overexpressing cells compared with the cells transfected with the control vector. Similarly, EcDDX41 overexpression also significantly decreased the transcription of CP and RdRp during RGNNV infection compared with the cells transfected with the control vector (Fig. 5B). Consistent with this finding, the protein synthesis of RGNNV CP and SGIV MCP were both significantly inhibited in EcDDX41-overexpressing cells (Supplemental Fig. 2). These findings indicate that EcDDX41 exerts antiviral activity against fish virus infection.

3.5. EcDDX41-overexpression evoked an interferon and inflammatory response

To elucidate the antiviral mechanism of EcDDX41 in response to fish virus infection, we investigated the effect of EcDDX41 on the host interferon and overall inflammatory response. As shown in Fig. 6A, the level of interferon signaling molecule expression, including IRF3, IRF7, IRF1, IRF2, MXI, and IFP35 were all significantly up-regulated in EcDDX41-overexpressing cells compared to those transfected with the

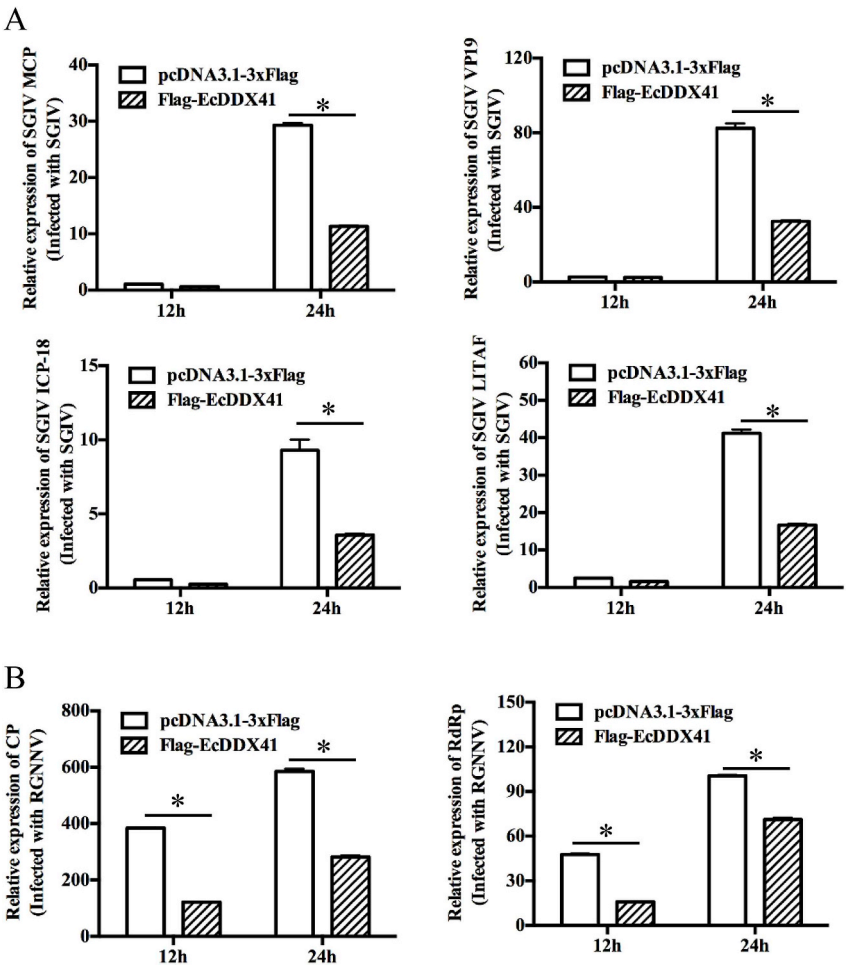

Fig. 5. EcDDX41 overexpression suppresses fish virus replication. EcDDX41 overexpression significantly decreased the levels of SGIV core gene expression, including MCP, VP19, ICP-18, and LITAF (A), as well as RGNNV CP and RdRp (B).

control vector. In addition, EcDDX41-overexpression also significantly increased the levels of pro-inflammatory factor expression, including TNFα, IL-1β, and IL-8 (Fig. 6B). Thus, we speculated that EcDDX41 was able to evoke both an interferon and inflammatory response during viral infection.

To further clarify whether other signaling molecules were involved in the EcDDX41-induced interferon response, we examined the levels of interferon signaling molecule expression in EcDDX41 and MAVS/MITA/TBK1 co-transfected cells. As shown in Fig. 7A, EcDDX41 overexpression significantly enhanced the level of IRF3, IRF7, ISG15 and MXII expression induced by MAVS. Similarly, in EcDDX41 and TBK1 co-transfected cells, the level of IRF3, IRF7, ISG15 and MXII expression was also significantly increased compared to the cells transfected with TBK1 alone (Fig. 7B). In contrast, no regulatory effects were detected in the EcDDX41 and MITA co-transfected cells (Fig. 8). Taken together, these results suggest that EcDDX41 was able to enhance the MAVS- and TBK1-induced interferon immune response *in vitro*.

4. Discussion

Substantial progress has been made regarding the function of DDX41 on infection and immunity in mammals; however, the function of fish DDX41 remains largely unknown [4,21]. To better understand the function of fish DDX41 against viral infection, we cloned the full-length cDNA of grouper DDX41 and investigated its role during SGIV and RGNNV infection.

The appropriate subcellular localization of proteins is critical because it provides the physiological environment for their function [22]. DDX41 was firstly characterized as a cytosolic protein in HEK293T and D2SC cells [4,23]. Interestingly, several studies demonstrate that DDX41 is located in the nucleus of murine lung fibroblasts, and THP1 cells [7,24]. In addition, DDX41 has also been observed to undergo nucleocytoplasmic shuttling in transfected CHO cells [25]. In teleost, recent studies show that zebrafish DDX41 is a trafficking protein distributed in the nucleus of unstimulated cells that is transported into the cytoplasm following DNA stimulation. Moreover, zebrafish DDX41 mRNA was highly expressed in brain and immune-relevant tissues, including liver and gill [9]. In the present study, our results indicate that EcDDX41 shares a 94% identity with zebrafish, and contains both the conserved DEAD and HELICc domains. In healthy grouper, EcDDX41 was predominantly observed in the gill, followed by the brain and liver, suggesting that EcDDX41 might exerts various physiological and immunological roles like zebrafish DDX41 [9]. Consistent with its endogenous expression, exogenous EcDDX41 was distributed only in the

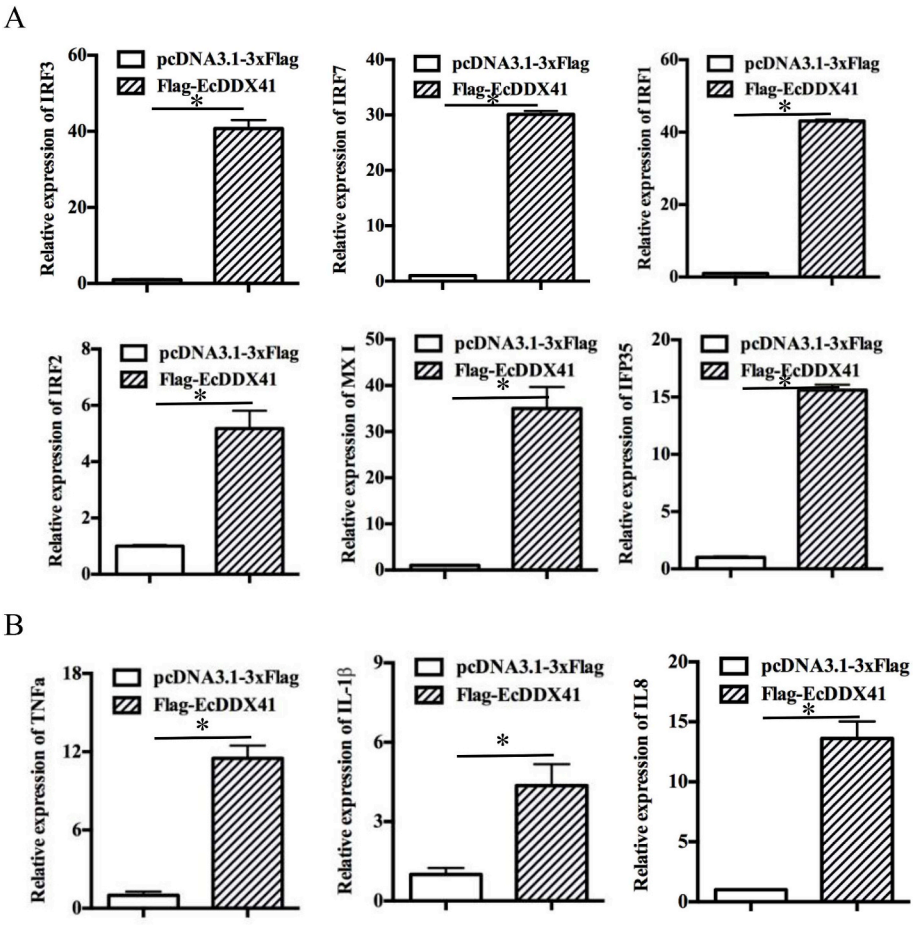

Fig. 6. EcDDX41 overexpression significantly evoked the interferon and inflammatory response. The expression levels of interferon signaling molecule (A), including IRF3, IRF7, IRF1, IRF2, MXI, and IFP35, as well as pro-inflammatory cytokine expression (B), including TNFα, IL-1β, and IL-8 were detected in EcDDX41 overexpressing cells. GS cells were transfected with Flag-EcDDX41 or pcDNA3.1-3xFlag at 800 ng/well, and the expression of the host factors were examined using qPCR.

nucleus of transfected grouper cells. Moreover, in SGIV infected cells, the localization of EcDDX41 was altered, and EcDDX41 was partly translocated from nucleus to cytoplasm, implying that EcDDX41 might exert play crucial roles in DNA virus infection.

An increasing number of studies have demonstrated that DDX41 plays an important role in the innate immune response of different species, including shrimp, fish, ducks, and humans [4,26,27]. Moreover, DDX41 is thought to function as an antiviral factor against virus infection. The replication of duck enteritis virus (DEV) *in vitro* is significantly inhibited in duck DDX41-expressed DEFs [26]. Additionally, the suppression of black tiger shrimp *Penaeus monodon* DDX41 (PmDDX41) by dsRNA-mediated gene silencing results in the more rapid death of WSSV-infected shrimp [27]. Here, we found that the transcription of EcDDX41 was both significantly up-regulated during SGIV and RGNNV infection. Although DDX41 is a DNA sensor, the up-regulation of DDX41 during RGNNV infection might be due to regulatory roles of the other activated molecules on DDX41. For example, TRIM21 could be activated by Japanese encephalitis virus, and TRIM21 also interacted with DDX41 [28,29]. Whether RGNNV infection activated some molecules and then regulated DDX41 expression needed further investigation. Further analysis showed that EcDDX41 overexpression in grouper cells significantly suppressed the viral gene transcription of SGIV and RGNNV, which suggests that EcDDX41 exerted antiviral activity against fish virus infection. Moreover, the overexpression of duck DDX41 triggers the activation of the expression of the transcription factors, NF-κB and IFN-β, in DEFs [26]. In addition, the overexpression of flounder DDX41 also induces the expression of antiviral and inflammatory cytokines via treatment with cytoplasmic C-di-GMP [8]. Our results also indicate that EcDDX41 overexpression in grouper cells significantly up-regulated the expression of interferon and inflammatory cytokines. Furthermore, we found that EcDDX41 enhanced the interferon response induced by MAVS and TBK1, but not MITA in co-transfected cells. Although the overexpression of both human DDX41 and MITA has a synergistic effect on promoting the interferon response, this might be due to the co-localization of DDX41 and MITA [4]. In zebrafish, DDX41 only significantly enhances MITA-induced interferon promoter activity in response to treatment with poly (dA:dT), but not PBS [9]. Thus, we speculate that EcDDX41 might also be able to enhance the MITA-induced immune response under stimulation with double-stranded DNA or bacterial cyclic dinucleotides. However, the detailed regulatory mechanism of DDX41 on MITA and their interaction should be further investigated.

In the current study, a DDX41 homolog from grouper was cloned and characterized. Our results showed that EcDDX41 was up-regulated during both SGIV and RGNNV infection in grouper cells. EcDDX41 was found to be primarily localized in the nucleus of grouper cells. Furthermore, EcDDX41 overexpression significantly inhibited SGIV and RGNNV replication, and significantly upregulated the expression of antiviral and inflammatory cytokine genes. In addition, EcDDX41 positively regulated the MAVS- and TBK1-induced interferon immune response. Taken together, we speculate that EcDDX41 mediates critical antiviral activity against grouper virus infection by positively regulating the interferon and inflammatory response.

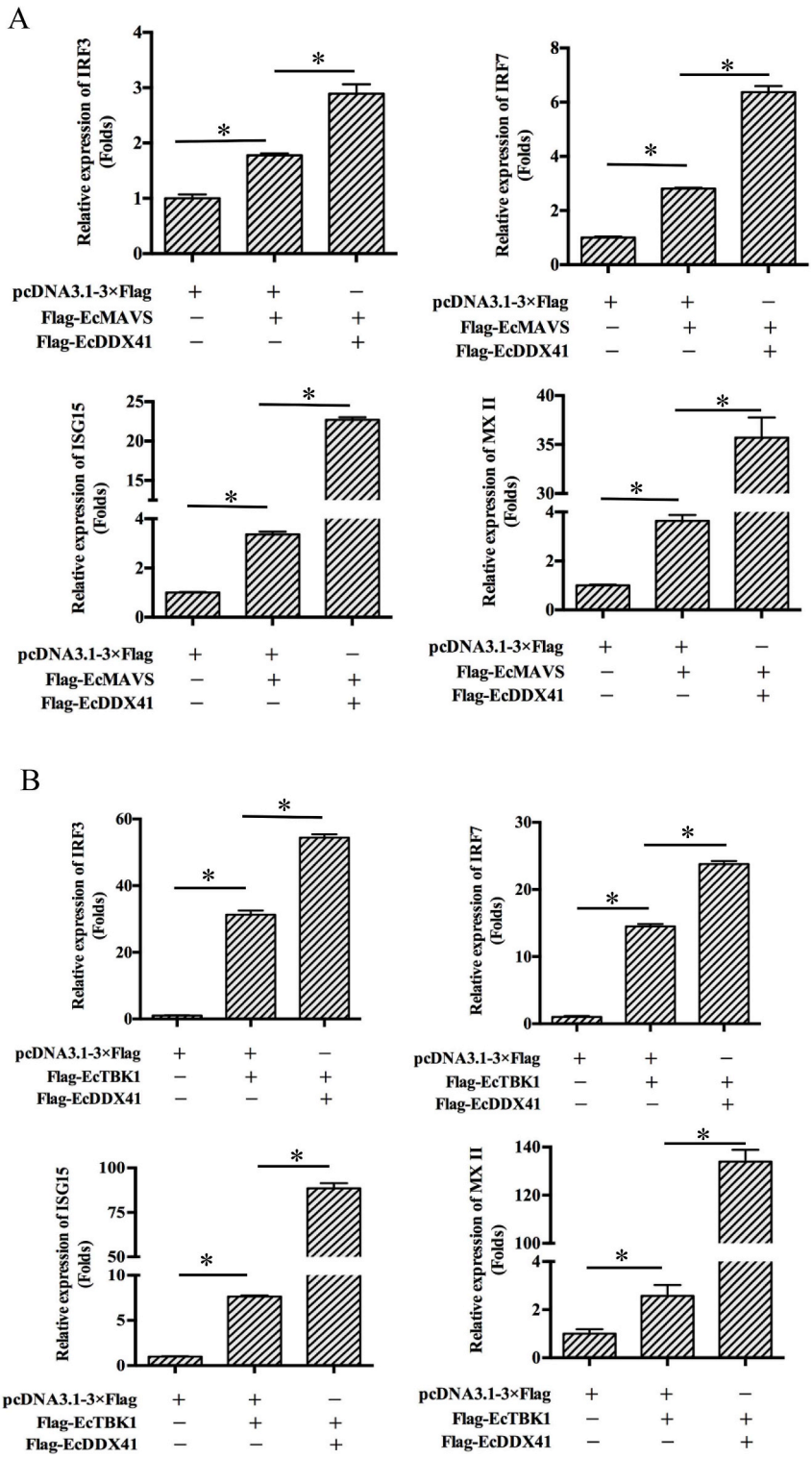

Fig. 7. EcDDX41 enhanced the interferon response induced by MAVS or TBK1. GS cells were co-transfected with EcDDX41 (400 ng/well) and MAVS (400 ng/well) (A) or TBK1 (400 ng/well) (B), then the level of interferon signaling molecule expression, including IRF3, IRF7, IRF1, IRF2, MXII, and ISG15 were examined using qPCR.

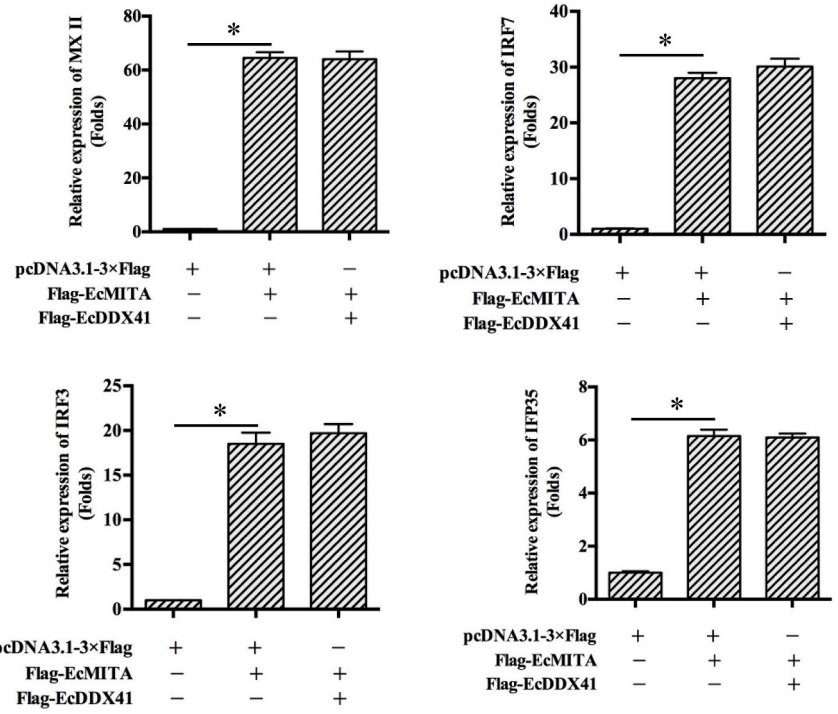

Fig. 8. EcDDX41 exhibited no effect on the MITA-evoked interferon response. In EcDDX41 (400 ng/well) and EcMITA (400 ng/well) co-transfected cells, the level of interferon signaling molecule expression, including IRF3, IRF7, IRF1, IRF2, MXII, and ISG15 was examined using qPCR.

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Appendix A. Supplementary data

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