Characterization of c-Jun from orange-spotted grouper, Epinephelus coioides involved in SGIV infection

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The nuclear phosphoprotein c-Jun is a member of the AP1 family of transcription activating complex, can be induced by various extracellular stimuli such as virus infection. In this study, the c-Jun gene (Ec-c-Jun) was cloned from orange-spotted grouper, Epinephelus coioides. The full-length Ec-c-Jun cDNA is composed of 2046 bp and encodes a polypeptide of 328 amino acids with 81% identity of zebrafish. Amino acid alignment analysis indicated that Ec-c-Jun contained three conserved domains including a transactivation domain (TAD), a DNA-binding domain (DBD) and leucine zipper domain (LZD). RT-PCR results showed that Ec-c-Jun transcript was most abundant in spleen, kidney, heart and gill. The expression of Ec-c-Jun was up-regulated after challenged with Singapore grouper iridovirus (SGIV). To investigate the roles of Ec-c-Jun during SGIV infection, we constructed its dominant-negative mutant (DN-Ec-c-Jun) by deleting the major TAD that lacks amino acids 3–122. Fluorescence microscopy observation revealed that Ec-c-Jun and DN-Ec-c-Jun were expressed predominantly in the nucleus in transfected cells. Interestingly, the green fluorescence of Ec-c-Jun was congregated and co-localized with virus assembly sites at the late stage of SGIV infection. However, in DN-Ec-c-Jun transfected cells, no virus assembly sites were observed, and the distribution of fluorescence remained unchanged. Moreover, overexpression of DN-Ec-c-Jun in vitro delayed the occurrence of CPE induced by SGIV infection and inhibited the virus gene transcription. In addition, ectopic expression of DN-Ec-c-Jun was able to inhibit SGIV induced c-Jun/AP1 promoter activity in GS cells. Thus, we proposed that c-Jun transcription factor was essential for SGIV replication in vitro. Our results will contribute to understanding the crucial roles of JNK signaling pathway in fish virus infection.

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1. Introduction

Transcription factor c-Jun is a member of the AP1 (activator protein one) complex consists of a dimer containing Jun (c-Jun, Jun-B and Jun-D), Fos (c-Fos, Fos-B, Fra-1, Fra-2), Atf (activating transcription factor) and Maf (musculoaponeurotic fibrosarcoma) protein families [1]. Jun and Fos proteins are DNA-binding proteins that are involved in the gene expression through transcriptional regulation, including modulating cell proliferation, tumor progression, cell death, in response to different biological stress signals [2–4]. Studies of c-Jun and related transcription factor substrates have provided clues about the additional docking domains recognized by JNK [5]. Increased literatures revealed that JNK signaling pathway is activated during bombyx mori nucleopolyhedrovirus, rotavirus, influenza virus (IV) and mouse hepatitis virus (MHV) infection, and regulated various inflammatory mediators, including cytokines and chemokine genes [6–9]. Moreover, the crucial effector molecule c-Jun also plays important roles in virus pathogenesis [10–14]. Suppression of c-Jun improved the survival rate of mice infected with H5N1 virus and decreased the CD8+ T cell proliferation in vivo compared with control groups [14]. Differently,
overexpression of either c-Jun could significantly inhibited major early regulatory protein large T-Ag-mediated JC virus (JCV) DNA replication, and further studies demonstrated that transcription factors c-Jun physically and functionally interacted with JCV T-Ag and that this interaction modulates JCV transcription and replication in glial cells [10]. Although the functions of c-Jun from mammals are well understood, few reports focused on c-Jun homologs from lower vertebrates [15]. To our knowledge, Japanese puffer fish, Fugu rubripes contained more numbers of Jun genes than mammals, but the functions of these Jun genes still remained unknown [16].

Recent studies have described that inhibitors can block transactivation, which are alternatively spliced forms of the specific transcription factors [17]. Dominant-negative c-Jun (DN-c-Jun) was resistant to NGF withdrawal-induced death and promoted the survival of sympathetic neurons [18]. DN-c-Jun was capable of suppressing tumor formation by carcinogen initiated malignant tumor cells in vivo [19]. DN-c-Jun inhibited AP-1 activity by dimerizing with wild-type AP-1 proteins to yield low-activity dimers containing only one transactivation domain. Transgenic expression of DN-c-Jun in the mouse skin inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced AP-1 transactivation and protected against both TPA-induced and E7-enhanced two-stage skin carcinogenesis [20,21]. Additionally, the activities of AP-1 and NFκB were inhibited in the expression of DN-c-Jun [22]. Ultimately, activation of transcription factors involved complicated signal transduction pathways by regulation of gene expression.

Orange-spotted grouper, Epinephelus coioides is widely cultured in China and Southeast Asian countries because of its excellent seafood quality and its high market value. However, outbreaks of diseases caused by virus pathogens, such as iridovirus and nervous necrosis virus, have affected the grouper aquaculture industry causing heavy economic losses [22,23]. Singapore grouper iridovirus (SGIV), a novel iridovirus belonging to the genus Iridoviridae, was isolated from diseased grouper [24]. Our previous studies demonstrated that MAPK signaling pathways played important roles during SGIV infection induced apoptosis [8,25]. Moreover, JNK signaling pathway modulated the inflammatory responses and was essential for SGIV replication. As a crucial downstream effector of JNK pathway, c-Jun phosphorylation could also be detected after SGIV infection [8]. In present study, the c-Jun gene was cloned from E. coioides and its roles during SGIV replication were investigated in vitro. Our findings will shed new light on the molecular mechanism of fish c-Jun during virus infection.

2. Material and methods

2.1. Fish, cells and virus

Juvenile orange-spotted grouper, E. coioides (50–60 g) were purchased from a fish farm in Huizhou, Guangdong province, China. Fish were acclimatized in a laboratory recirculating seawater system at 25–30 °C and fed twice daily for two weeks before experimental manipulation.

The fish were anesthetized using “cold-anesthetization” by adding ice into fish bucket before killing. A series of tissue samples including liver, spleen, kidney, head kidney, gill, brain, intestine, skin, stomach, heart and muscle were collected from the killed fish and immediately frozen by liquid nitrogen, followed by storage at −80 °C until used. Grouper spleen cells (GS) were grown in Leibovitz’s L15 medium that contained 10% fetal bovine serum (Invitrogen, USA) at 25 °C [26]. SGIV was kept in our laboratory. Propagation of SGIV was performed as described previously [27].

2.2. Cloning and sequencing of E. coioides c-Jun (Ec-c-Jun)

Total RNA was extracted from head kidney of E. coioides tissues using TRizol Reagent (Invitrogen) according to the manufacturer’s protocol. The quality of total RNA was detected by electrophoresis on 1% agarose gel. The RNA was used for rapid amplification of cDNA ends (RACE-PCR) and cDNA synthesis ReverTra Ace (TOYOBO, Japan). The first-strand cDNA was synthesized from total head kidney RNA with the SMARTTM RACE cDNA amplification kit (Clontech, USA) following the manufacturer’s protocol for 5′ RACE and 3′ RACE. The primers used for RACE PCR (Table 1) were designed based on the identified expressed sequence tag (EST) sequence from the transcriptome library established in our laboratory (Accession No. SRA040065.1) [28]. In detail, the gene specific primer c-Jun 5′NGSP1, c-Jun 3′GSP1 (Table 1) and UPM (supplied by the kit) were used for the first round PCR. The PCR was conducted at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 68 °C for 30 s and 72 °C for 50 s. The product of the first round PCR was diluted 10 times and then used as the template for the nested PCR. The nested PCR was performed with the gene specific primers c-Jun 5′NGSP2, c-Jun 3′GSP2 (Table 1) and UPM (supplied by the kit). The RACE PCR condition and assembly of Ec-c-Jun cDNA were performed as described previously [27].

2.3. Construction of dominant-negative mutant c-Jun gene (DN-Ec-c-Jun)

To develop a dominant-negative mutant of c-Jun, we mutated the transactivation domain (TAD) of c-Jun to produce the mutant (DN-Ec-c-Jun) [29]. The coding sequence corresponding to the Ec-c-Jun was amplified with PCR primers pEGFP-c-Jun-F (BglII site is underlined) and pEGFP-c-Jun-R (SalI site is underlined) and pEGFP-c-Jun-F and pEGFP-c-Jun-R (SalI site is underlined) (Table 1). The coding sequence corresponding to the DN-Ec-c-Jun was amplified with PCR primers pEGFP-DN-c-Jun-F and pEGFP-DN-c-Jun-R (SalI site is underlined) and pEGFP-c-Jun-R (SalI site is underlined) (Table 1). The Ec-c-Jun and DN-Ec-c-Jun were produced using PCR amplify, and sequenced to ensure that the PCR amplification did not introduce any mutations. Assembly of the plasmids was performed on the basis of vector pEGFP-N3 (Clontech, USA).

<table>
<thead>
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2.4. Bioinformatics analysis

The cDNA and predicted amino acid sequences of Ec-c-Jun were analyzed using Genetyx7.0 software. The similarity of Ec-c-Jun with other c-Juns were analyzed using the BLASTP search program at the NCBI (www.ncbi.nlm.nih.gov/blast). Multiple-sequence alignment of the reported c-Jun amino acid sequences was performed using ClustalX2.0 and a phylogenetic tree was constructed using the MEGA 5.0 software, numbers at branch nodes represent the bootstrap majority consensus values of 1000 replicates.

2.5. Tissue distribution of Ec-c-Jun mRNAs

Total RNA was extracted from healthy orange-spotted grouper liver, spleen, trunk kidney, head kidney, gill, brain, intestine, skin, stomach, heart and muscle with TRIzol Reagent (Invitrogen, USA) according to manufacturer’s protocol. Expression levels of Ec-c-Jun in different tissues were determined by RT-PCR using primers RT-Actin-F and RT-Actin-R to amplify the internal control β-actin while RT-c-Jun-F and RT-c-Jun-R were used to amplify Ec-c-Jun (Table 1). The PCR conditions were 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, with a final elongation step of 10 min at 72 °C.

2.6. Virus challenge and qPCR

In order to determine the expression of Ec-c-Jun in grouper spleen against immune challenges, six groups of fish were i.p. injected with SGIV and the expression patterns of Ec-c-Jun was investigated by qPCR at different time points of 0, 6, 12, 24, 48, 72 and 96 h post infection. For the virus challenge experiment, each grouper was intraperitoneal (ip) injected with 200 TCID50, and each control sample was ip injected with 200 μl PBS respectively. Six fish in each group were collected for RNA extraction and qPCR at 0, 6, 12, 24, 48, 72 and 96 h of post-injection. Expression levels of Ec-c-Jun were examined by qPCR in spleen after challenging with SGIV. In detail, qPCR was carried out using a LightCycler® 480 Real-Time PCR System (Roche, Basel, Switzerland), with SYBR Green as the fluorescent dye, according to the manufacturer’s protocol (TOYOBO). Each of the samples contained six independent individuals respectively to eliminate the individual differences. β-actin was Amplified as an internal control using primers RT-Actin-F and RT-Actin-R, while RT-c-Jun-F and RT-c-Jun-R were used to amplify Ec-c-Jun (Table 1). All primer pairs amplified a single PCR product with the expected size using the similar Tm value by agarose gel electrophoresis and melting curve analysis. The PCR amplification efficiency of each primer pair is normal and identical according to methods described previously [30]. All samples at indicated time points after infection were analyzed in triplicate wells using the cycling condition as follows: 94 °C for 5 min, followed by 40 cycles of 5 s at 94 °C, 10 s at 60 °C and 15 s at 72 °C. We analyzed the relative gene expression by the typical Ct method (2- ΔΔCt method) [31], then the results were calculated as the folds based on the expression level of Ec-c-Jun in different challenged grouper relative to that in PBS injected grouper at the same time point. Data were expressed as mean ± SD, and statistic analysis were performed using SPSS software.

To detect whether the transcription levels of viral genes were altered after overexpression of DN-Ec-c-Jun, qRT-PCR was used to evaluate the relative RNA expression of SGIV genes encoding structural proteins, including the major capsid protein (ORF 072), envelope proteins (ORF 016), an early transcript (ORF049), an immediate-early gene (ORF 162). The procedures were performed as described above.

2.7. Fluorescence microscopy

The subcellular localizations of Ec-c-Jun and DN-Ec-c-Jun were determined by Ec-c-Jun-GFP and DN-Ec-c-Jun fusion protein expressions in GS cells. The GS cells were seeded onto coverslips (10 mm × 10 mm) in a 24-well plate. After the cell adhering for 18 h, the EAGS cells were transiently transfected with empty pEGFP-C1 and recombinant pEGFP-Ec-c-Jun pEGFP-DN-Ec-c-Jun using LipofectamineTM 2000Reagent (Invitrogen, USA) according to the manufacturer’s protocol. After transfection for 24 h, the GS cells were infected with SGIV 24 h. The coverslips were fixed with 4% paraformaldehyde for 30 min, and then stained with 6-diamidino-2-phenylindole (DAPI) (1 μg/ml) for 15 min. Finally, the cells were rinsed with PBS, mounted with 50% glycerol, and observed using fluorescence microscopy (Leica, Germany).

2.8. Reporter gene assay

GS cells were seeded to 24-well plates for 18 h, then transiently transfected with c-Jun luciferase reporter construct (1 μg/well) and pCMV-β-gal reporter construct (0.4 μg/well) (Clontech), and we also co-transfected with c-Jun luciferase reporter vector (0.6 μg/well) or AP1 luciferase reporter vector (0.6 μg/well), pEGFP-DN-Ec-c-Jun (0.4 μg/well), and pEGFP-C1 as a control, using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. After an additional 18 h of incubation, cells were mock infected or infected with SGIV and incubated for various intervals. Samples were prepared for a luciferase assay using a commercial Luciferase Assay System (Promega). Briefly, cells were harvested and lysed in lysis buffer. Cells were vortexed and centrifuged at 14 000 g for 5 min. The supernatants were added to 100 μl luciferase assay buffer, and luminescence was measured immediately using a Victor X5 Multilabel plate reader (PerkinElmer). The results were representative of three independent experiments, and each independent experiment was performed in triplicate.

2.9. Statistical analysis

All analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). All data were expressed as mean relative stimulation as means ± SD from three independent experiments with each performed in triplicate wells, and then subjected to Student’s t-test. Differences were considered statistically significant at P < 0.05.

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**Table 2**

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3. Results

3.1. Characterization of Ec-c-Jun

The cDNA sequence of Ec-c-Jun was deposited in Genbank under accession no. KF366905. The full length cDNA of Ec-c-Jun is of 2046 bp, containing a 5'-terminal untranslated region (UTR) of 317 bp, a 3'-terminal UTR of 745 bp with a 21 bp poly (A) tail and a consensus polyadenylation signal sequence AATAAA, and an open reading frame (ORF) of 984 bp encoding a polypeptide of 328 amino acids with a predicted molecular mass of 36 kDa and a theoretical isoelectric point of 8.72. The deduced amino acid sequence of Ec-c-Jun contains a putative signal peptide of 15 residues at the N-terminal of deduced protein (Fig. 1). Bioinformatics analysis showed that Ec-c-Jun contained four N-glycosylation sites, one Protein kinase C phosphorylation site, seven Casein kinase II phosphorylation sites, two N-myristoylation sites, one Leucine zipper pattern, one Basic-leucine zipper (bZIP) domain signature. The deduced amino acid sequence of Ec-c-Jun was aligned with other known c-Juns and several important domains such as transactivation domain (TAD), DNA-binding domain (DBD) and Leucine zipper domain (LZD) were found in the Ec-c-Jun (Fig. 2). Phylogenetic tree was constructed based on amino acid sequences of c-Jun from various species by the Neighbor-Joining method. Ec-c-Jun presented the closest distant relationship with c-Jun of zebrafish, Danio rerio (Fig. 3).

3.2. Expression patterns of Ec-c-Jun

The RT-PCR results showed the expression of Ec-c-Jun mRNA in various normal tissues using β-actin as a reference gene. Ec-c-Jun transcript was abundant in the head kidney, gill, brain, and to a less extent in the stomach, intestine, trunk kidney, spleen, skin, liver (Fig. 4A).

The results of qPCR analysis revealed that the transcripts of Ec-c-Jun were up-regulated by SGIV infection, and reached the peak at 48 h and 72 h post-infection. In detail, after SGIV challenge, Ec-c-Jun transcripts in grouper spleen increased up to 3-fold at 48 h and reached up to 5-fold at 72 h, then gradually decreased to original level at 96 h post-infection compared with the control fish. All these data indicated that Ec-c-Jun might be activated and play critical role during SGIV infection (Fig. 4B).

3.3. Subcellular localization of Ec-c-Jun

As shown in Fig. 5, the green fluorescence in Ec-c-Jun-GFP and DN-Ec-c-Jun fusion protein transfected GS cells was distributed mainly in the nucleus (Fig. 5A, lower row). In pEGFP-C1 transfected cells, the fluorescence signal was observed in both cytoplasm and nucleus (Fig. 5A, upper row). In the late stage of SGIV infection (24 h p.i.), the green fluorescence (in web version) was observed in the virus assembly sites with Ec-c-Jun (Fig. 5B, upper row). However, no change with DN-Ec-c-Jun was observed, and the formed virus assembly site was also not observed (Fig. 5B, lower row).

3.4. The roles of Ec-c-Jun in SGIV replication in vitro

To determine the roles of Ec-c-Jun in SGIV replication, the GS cells were transfected with DN-Ec-c-Jun and then infected with SGIV. The expression of green fluorescence (in web version) was observed under fluorescence microscopy (Fig. 6A). After SGIV infection, we found that the CPE progression induced by virus was inhibited significantly in DN-Ec-c-Jun overexpressing cells (Fig. 6B). We also detected the effects of overexpression of DN-Ec-c-Jun on viral gene transcription by qPCR. As shown in Fig. 6C, the transcripts of 4 viral genes, including “IE” gene (ORF049), “E” gene (ORF049) and “L” genes (ORF072 and ORF075) were all significantly down-regulated by overexpression of DN-Ec-c-Jun. Thus, we proposed that Ec-c-Jun was essential in SGIV replication in vitro.

3.5. Effects of DN-Ec-c-Jun on c-Jun and AP1 promoter activity

Our previous studies showed that AP-1 promoter activity was increased during SGIV infection [8]. Here, we also detected the promoter activity of c-Jun during SGIV infection using luciferase reporter gene assays. As shown in Fig. 7A, Time-dependent promoter activity of c-Jun increased significantly after SGIV infection. In detail, SGIV infection resulted in approximate increases of 5.27-fold in c-Jun activation at 48 h p.i. To evaluate whether SGIV induced c-Jun or AP1 promoter activity was regulated by Ec-c-Jun, we transfected GS cells with DN-Ec-c-Jun. We found that DN-Ec-c-Jun overexpression decreased SGIV induced c-Jun promoter activity up to 66% (Fig. 7B), while the AP1 activity was decreased to 69% (Fig. 7C), compared with the control cells. These results suggested that SGIV infection activated c-Jun, and DN-Ec-c-Jun inhibited the activity of c-Jun and AP1 promoter.

4. Discussion

c-Jun N-terminal kinases (JNK), a classic pathway involved in numerous cell activities, such as proliferation, apoptosis and inflammation, and are activated in course of many viral infection [32,33]. c-Jun is a downstream molecule of JNK and a crucial factor of activator protein AP1, may participate in virus infection. However, the role of c-Jun in SGIV infection has not been reported yet. In this study, we cloned the full-length cDNA of the c-Jun gene from orange-spotted grouper Epinephelus coioides, and characterized its roles by dominant-negative mutant during SGIV infection.

c-Jun is a nuclear protein that forms homodimers or heterodimers which then bind DNA in a sequence-specific manner [34]. The domains of c-Jun are necessary for c-Jun induced transcriptional activation, including a transactivation domain (TAD), DNA-binding domain (DBD), and a Leucine zipper domain (LZD) [17]. Our studies revealed that Ec-c-Jun encoded 328 amino acids protein and shared 81% identity to zebrafish. Although Ec-c-Jun only showed 70% identity to human, it contained conserved functional domains of c-Jun, including TAD, DBD and LZD, suggested that Ec-c-Jun might exert similar functions to mammalian c-Juns. After SGIV infection, the transcript of Ec-c-Jun in spleen was increased significantly in comparison to control. Moreover, the ectopic expressed Ec-c-Jun was translocated from nucleus to virus assembly sites. Given that virus assembly sites formed in iridovirus infection always contained numerous viral structural proteins, including membrane proteins and capsid proteins [35], we proposed that Ec-c-Jun might participate in SGIV infection via interaction with these viral proteins. During JCV infection, the transcription factors c-Jun physically and functionally interacted with JCV T-Ag and their interaction mediated JCV transcription and replication in giall cells [10]. The interaction of Ec-c-Jun and viral proteins during SGIV infection will be explored in the further study.

Increased reports demonstrated that JNK signaling pathway was crucial for virus infection [36–38]. As an important molecule in JNK signaling pathway, c-Jun also play important roles in virus pathogenesis [10,11]. During SGIV infection, JNK signaling pathway was activated, and phosphorylated c-Jun in nucleus increased significantly during the early stage of SGIV infection. Interestingly, the phosphorylated c-Jun was translocated into cytoplasm, and aggregated into virus assembly sites at the late stage of infection [8]. Given that the transactive activity could be inhibited by the DN-c-Jun [39,40], we constructed the deletion
Fig. 1. The nucleotide and deduced amino acid sequences of Ec-c-Jun. The translation start (ATG) and stop (TGA) codons were in bold. The mRNA instability motif (ATTTA) was underlined and the polyadenylation signal (AATAAA) was boxed.
Fig. 2. Multiple-sequence alignment of the deduced amino acid sequence of Ec-c-Jun with other known c-Juns. Sequences were aligned by clustalX2.0. Completely conserved residues across all species aligned were shaded in black. The predicted Transactivating Domain, DNA Binding Domain and Leucine Zipper were boxed. The Genbank accession numbers of the aligned c-Jun sequences were listed in Table 2.
mutant DN-Ec-c-Jun in this study. Our findings showed that overexpression of DN-Ec-c-Jun delayed the occurrence of CPE induced by SGIV infection and inhibited the virus gene transcription. Moreover, overexpression of DN-Ec-c-Jun suppressed the promoter activity of c-Jun or AP1 induced by SGIV infection. We speculated that DN-Ec-c-Jun might inhibit virus replication by decreasing the endogenous c-Jun and AP-1 transcriptional activity during SGIV infection. As an important member of MAPK family, p38MAPK was also demonstrated to be involved in SGIV infection [8]. In vitro, overexpression of Ec-p38 affected the occurrence of CPE and SGIV infection induced apoptosis in FHM cells [25]. It has been reported that p38 MAPK and c-Jun not only played important roles in apoptosis [3,41], but also could cooperate to regulate gene expression [42]. However, whether Ec-c-Jun and Ec-p38MAPK co-

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**Fig. 3.** Phylogenetic analysis of Ec-c-Jun with other reported c-Juns. The phylogenetic tree of the alignment amino acid sequences was constructed by neighbor-joining method within MEGA 5. The scale bar indicates the branch length, and the bootstrap confidence values were shown at the nodes of the tree. The GenBank accession numbers of the analyzed c-Jun sequences are listed in Table 2.

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**Fig. 4.** Analysis of the expression profiles of Ec-c-Jun. (A) Detection of Ec-c-Jun mRNA from various grouper tissues by RT-PCR. (B) Temporal expression analysis of Ec-c-Jun mRNA in the spleen of grouper challenged with SGIV. β-actin was amplified as an internal control. The changes in expression of each target gene were calculated as the folds based on the expression level of the c-Jun gene in SGIV challenged grouper relative to that in PBS injected grouper at the same time point. Data were expressed as mean ± SD (n = 6), and significant differences of the c-Jun gene expression between the challenged and control samples were indicated with an asterisk (*) at P < 0.05.
regulated SGIV replication and virus induced apoptosis needed further investigation.

In summary, we have successfully cloned and characterized the c-Jun gene from *E. coioides*. The expression of Ec-c-Jun was distributed predominantly in the nucleus, and mostly co-localized with virus assembly sites during SGIV infection. Inhibition of c-Jun transaction by overexpression of DN-Ec-c-Jun not only significantly reduced viral gene replication and virus assembly, but also

Fig. 5. The subcellular localization of Ec-c-Jun and DN-Ec-c-Jun in GS cells. (A) Distribution of Ec-c-Jun and DN-Ec-c-Jun in GS cells. (B) The response of Ec-c-Jun and DN-Ec-c-Jun to SGIV infection. After SGIV infection, cells were fixed. Nuclei were stained using DAPI. The arrows indicated viral assembly sites in SGIV infected cells.
Fig. 6. Effects of DN-Ec-c-Jun overexpression on viral gene transcription. (A) The expression of DN-Ec-c-Jun was confirmed in vector or DN-Ec-c-Jun transfected cells using transfection. (B) After transfection, cells were infected with SGIV for 24 and 48 h, respectively. (C) Total RNA of infected-cells or mock-cells were extracted, and the mRNA level of SGIV gene was detected by qRT-PCR, respectively. All data were normalized relative to β-actin and represented by means ± SD (n = 3). *, P < 0.05.
inhibited virus induced c-Jun and AP1 promoter activation. Together, our current findings may provide the new insight into the understanding of the potential roles of the c-Jun in the pathogenesis of fish iridovirus.

Acknowledgments

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Fig. 7. c-Jun and AP1-dependent luciferase expression in SGIV infected G5 cells. (A) Cells were cotransfected with 0.4 μg pcMV-β-gal and 1 μg of the luciferase reporter construct c-Jun; (B) 0.6 μg c-Jun luciferase reporter vector and 0.4 μg pEGFP-DN-Ec-c-Jun or pEGFP-C1; (C) 0.6 μg AP1 luciferase reporter vector and 0.4 μg pEGFP-DN-Ec-c-Jun or pEGFP-C1, then infected with SGIV and incubated for the indicated time intervals. Cell extracts were prepared and luciferase (LUC) activity was measured. β-Galactosidase activity was detected to normalize for transfection efficiency. Experiments were carried out independently three times. Results are shown as means ± SD; *P < 0.05; **P < 0.01.


