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A Kruppel-like factor from *Macrobrachium rosenbergii* (MrKLF) involved in innate immunity against pathogen infection

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ABSTRACT

Kruppel-like factors (KLFs) belong to a family of zinc finger-containing transcription factors that are widely present in eukaryotes. In the present study, a novel KLF from the giant river prawn *Macrobrachium rosenbergii* (designated as *MrKLF*) was successfully cloned and characterized. The full-length cDNA of *MrKLF* was 1799 bp with an open reading frame of 1332 bp that encodes a putative protein of 444 amino acids, including three conserved ZnF_C2H2 domains at the C-terminus. Multiple alignment analysis showed that MrKLF and other crustacean KLFs shared high similarity. Quantitative real-time PCR analysis revealed that *MrKLF* mRNA was found in different tissues of prawns and detected in the gills, hepatopancreas, and intestines. After the challenge with *Vibrio parahaemolyticus* and *Aeromonas hydrophila*, different expression patterns of *MrKLF* in the gills, intestines, and hepatopancreas were observed. RNA interference analysis indicated that *MrKLF* was involved in regulating the expression of four antimicrobial peptides, namely, *Crustin* (*Crus*) *2*, *Crus8*, *anti-lipopolysaccharide factor* (*ALF*) *1*, and *ALF3*. These results help promote research on *M. rosenbergii* innate immunity.

1. Introduction

Kruppel-like factors (KLFs) belong to a family of zinc finger-containing transcription factors, which are widely present in eukaryotes [1]. KLFs are highly homologous to the embryonic regulatory gene *Kruppel* in *Drosophila melanogaster* [2], which serve functions in hematopoiesis, differentiation, cellular development, metabolism, and activation [3,4]. KLFs share within their C-terminal regions three highly conserved Cys2–His2 zinc finger (ZnF_C2H2) DNA-binding domains that recognize either CACCC-elements or GC-boxes in the promoter of target genes [1,5,6]. The sequence in the N-terminal domains of KLFs are much more variable; it allows KLFs to participate in different biological processes [7,8].

To date, 18 predicted mammalian KLFs have been expressed in various tissues and during periods of development, although the *KLF18* gene is likely a pseudogene [9,10]. KLFs commonly function as transcriptional activators or repressors [1,5]. For example, KLF6 acts as a co-activator of NF-κB and is crucial for the transcription of selected downstream genes mediated by NF-κB after TNFα and IL-1β stimulation [11]. Through its binding to the transcriptional coactivator CBP/p300, KLF1 interacts with a SWI/SNF-like remodeling complex to regulate tissue-specific expression of β-globin [12]. KLF2 acts as a regulator of inflammation associates with CBP/p300, which alters the ability of KLF2 to prevent the interaction of NF-κB/p300 and subsequent activation of the vascular cell adhesion molecule-1 (VCAM-1) promoter [13]. KLF8 as a CACCC-box binding protein interacts with the co-repressor protein CtBP through a N-terminal Pro-Val-Asp-Leu-Ser motif and represses transcription [14]. KLF3 is located in the regulatory regions of the inflammatory modulator galectin-3 *Lgals3* gene; KLF3 directly binds its homologous elements (CACCC boxes) in the galectin-3 promoter and represses its activation in cellular analysis [15]. Upon virus infection, KLF4 is translocated from cytosol to nucleus and binds to the promoter of *IFNB* gene, thereby inhibiting the recruitment of IRF3 to the *IFNB* promoter [16]. Whether and how other KLFs function in invertebrate signaling are currently unknown.

The giant freshwater prawn, *Macrobrachium rosenbergii*, is an important economic aquaculture species in China and other Southeast Asian countries [17,18]. Nonetheless, prawn culture suffered a major

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setback, owing to the epidemic of infectious diseases caused by bacterial and viral pathogens [19,20]. Research on the immune defense mechanisms of prawns is desirable for disease control and to ensure long-term survival of crustacean farming.

In this study, we found that a new KLF family member *MrKLF* from *M. rosenbergii*. *MrKLF* was expressed in all examined tissues. Its mRNA level in gills, intestine, and hepatopancreas changed significantly when challenged by bacteria. In addition, knockdown of *MrKLF* by dsRNA interference inhibited the transcription of four *antimicrobial peptide* (*AMP*) genes. Our findings demonstrate that *MrKLF* is involved in the innate immunity of prawn in response to pathogens.

2. Materials and methods

2.1. Experimental animals and immune challenge

Two hundred *M. rosenbergii* (body weight: 15–20 g) were purchased from the local aquatic market in Nanjing, Jiangsu Province, China. The prawns were cultured in glass tanks with air-pumped freshwater at 25 °C for 1 week before the start of the experiments. *Vibrio parahaemolyticus* and *Aeromonas hydrophila* were kept in our laboratory. Hemolymph was extracted from five untreated prawns by using a 1 mL sterilized syringe preloaded with 1/2 volume of ice-cold anticoagulant citrate dextrose solution B (ACD-B, glucose, 1.47 g; citrate, 0.48 g; sodium citrate, 1.32 g; prepared in double distilled water at 100 mL final volume, and filtered with 0.22 μM filter). The buffer was prepared in double-distilled water and brought to 100 mL (pH 7.3). The extracted hemolymph was centrifuged immediately at 2000 rpm for 15 min at 4 °C to isolate the hemocytes. Other tissues, such as heart, hepatopancreas, gills, stomach, intestine, and muscle were also collected for tissue distribution analysis. The expression patterns of *MrKLF* were investigated after challenge with 50 μL of *V. parahaemolyticus* (3×10^6 cells) or *A. hydrophila* (3×10^5 cells), which was injected into the base of the abdominal segment of prawns. The corresponding control was challenged with 100 μL of sterile PBS (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4; pH 7.4). At 0, 2, 6, 12, and 24 h post-injection, the gills, intestines, and hepatopancreas were collected from five random prawns. All samples were stored at −80 °C for subsequent RNA extraction, and the experiments were performed in triplicate.

2.2. Total RNA isolation and cDNA synthesis

Total RNA was extracted from above tissues using RNApure High-Purity Total RNA Rapid Extraction Kit (Spin-column; Bioteke, Beijing, China) according to the manufacturer's instructions, and genomic DNA was removed by DNase I (Takara). RNA quality was assessed by electrophoresis on 1.2% agarose gel, and RNA concentrations were determined using a NanoDrop 2000 UV Spectrophotometer (Thermo Fisher). To synthesize the first-strand cDNA for RACE reactions, 2 μg RNA from hepatopancreas was used as template and reverse transcribed using a SMARTer™ RACE cDNA Amplification Kit (Clontech, USA) following the manufacture's protocol. For real-time PCR analysis, first-strand cDNA synthesis was conducted with 1 μg RNA using PrimeScript® 1st Strand cDNA Synthesis Kit (Takara, Japan) with Oligo-d(T) Primer.

2.3. cDNA cloning of MrKLF

To clone *MrKLF*, the partial cDNA sequence of this gene was extracted from our previous high-throughput transcriptome database. With specific primers (MrKLF-F: 5′-GAGCCTCTCAAATCTCCCCCACAT CGT-3′, MrKLF-R: 5′-ATGAACTTGGCACTACAGGAGGGGCAG-3′), the complete *MrKLF* cDNA was amplified by 5′ and 3′ RACE using Advantage® 2 PCR Kit (Clontech, USA). The following program was used for PCR amplification: 5 cycles at 94 °C for 30 s, and 72 °C for 3 min; followed by 5 cycles at 94 °C for 30 s, 70 °C for 30 s, and 72 °C for 3 min; and 20 cycles at 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min. All PCR products were analyzed by 1% agarose gel electrophoresis, and the PCR products were purified using a DNA gel extraction kit (Shanghai Generay Biotech Co., Ltd.), ligated with the pEasy-T3 vector (TransGen Biotech, China), and transformed into *Escherichia coli* Trans1-T1 cells. The positive recombinant clones were identified by PCR screening with M13F and M13R primers and by sequencing (Springen, China).

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ccccagccaggcaggcactccagtgacagtctcctctccactcgctcgcactctcacttac
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ggaatattcaccatgaagaagcaaatcatgtacaaacattcattgccttcaatgcaagat
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Fig. 1. Nucleotide and deduced amino acid sequence of MrKLF from *M. rosenbergii*. The low complexity regions are underlined. Three ZnF_C2H2 domains are shaded gray.

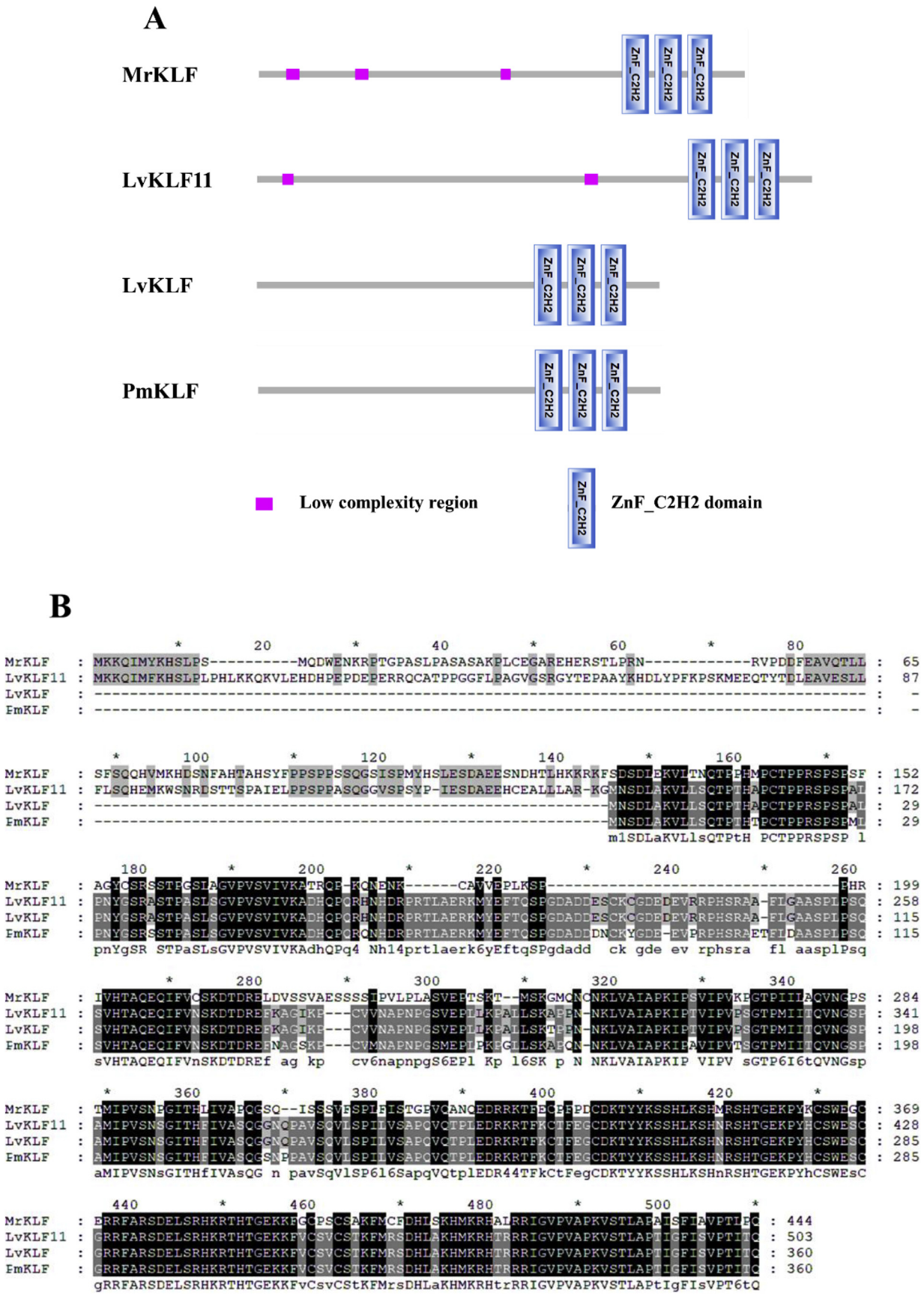

Fig. 2. (A) Domain organizations of MrKLF, LvKLF11, LvKLF, and PmKLF. (B) Multiple alignments of the deduced amino acid sequences of related KLFs. Identical residues are highlighted in black, and similar residues are highlighted in gray. The sequences are taken from GenBank, including LvKLF11 (No. AYM26544.1), LvKLF (No. AIE48080.1), and PmKLF (No. AEI83865.1).

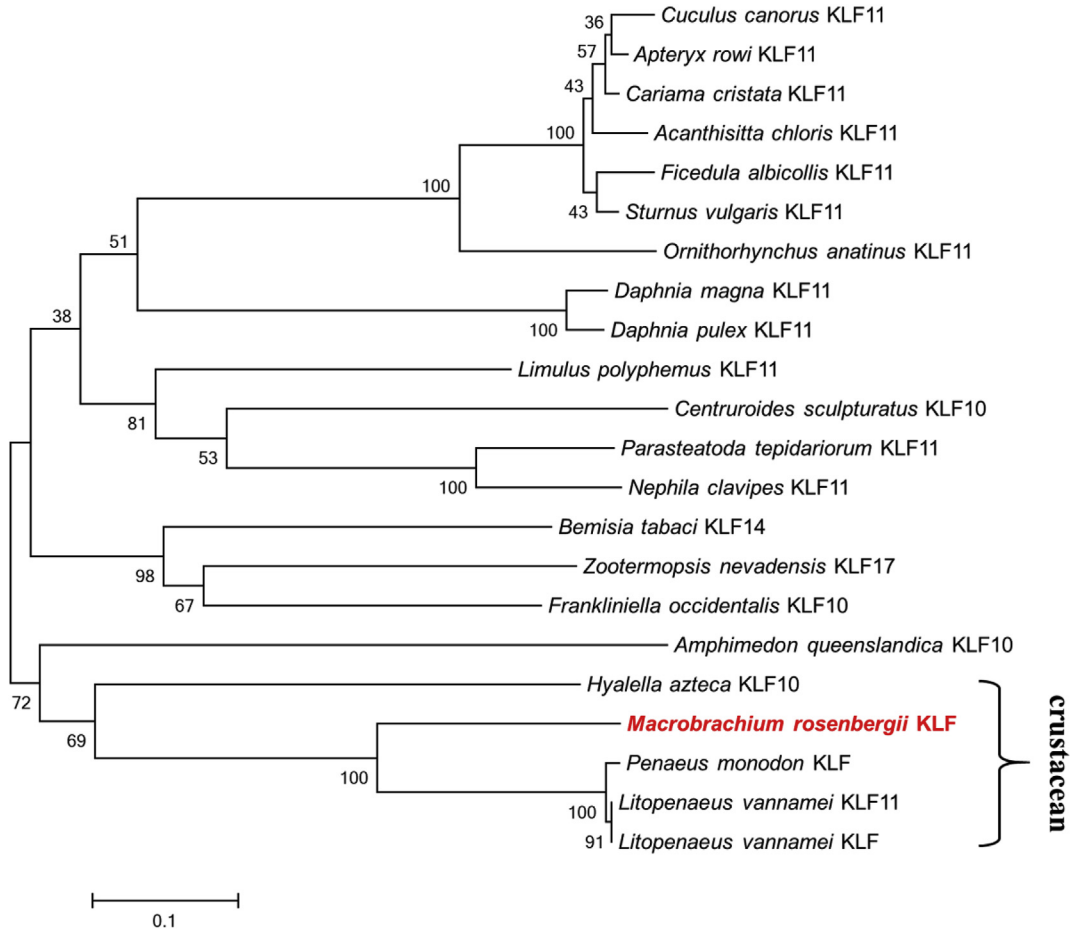

Fig. 3. Phylogenetic tree analysis of MrKLF and other closely related KLFs. *M. rosenbergii* KLF (MrKLF) is marked in red. *L. vannamei* KLF11: No. AYM26544.1; *L. vannamei* KLF: No. AIE48080.1; *P. monodon* KLF: No. AEI83865.1; *Daphnia magna* KLF11: No. KZS11418.1; *D. pulex* KLF11: No. EFX87222.1; *Limulus polyphemus* KLF11: No. XP_013775609.1; *Centruroides sculpturatus* KLF10: No. XP_023234801.1; *Hyalella azteca* KLF10: No. XP_018010554.1; *Amphimedon queenslandica* KLF10: No. XP_003387874.1; *Parasteatoda tepidariorum* KLF11: No. XP_015915498.1; *Bemisia tabaci* KLF14: No. XP_018899642.1; *Ficedula albicollis* KLF11: No. XP_005044345.1; *Nephila clavipes* KLF11: No. PRD30373.1; *Zootermopsis nevadensis* KLF17: No. XP_021928035.1; *Frankliniella occidentalis* KLF10: No. XP_026278851.1; *Cariama cristata* KLF11: No. XP_009695671.1; *Ornithorhynchus anatinus* KLF11: No. XP_007671524.1; *Acanthisitta chloris* KLF11: No. XP_009077667.1; *Cuculus canorus* KLF11: No. XP_009557968.1; *Sturnus vulgaris* KLF11: No. XP_014750475.1; and *Apteryx rowi* KLF11: No. XP_025932791.1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Bioinformatics analysis

Identification of MrKLF and other KLFs was performed using the online software BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The amino acids sequence was translated by ExPASy translate tool (http://web.expasy.org/translate/). Domain organization was identified by SMART (http://smart.embl-heidelberg.de/). The ClustalX 2.0 program (http://www.ebi.ac.uk/tools/clustalw2) and the GENEDOC software were used to carry out multiple alignments of amino acid sequences. The theoretical isoelectric point (pI) and molecular weight (MW) were calculated using the online ExPASy Compute pI/Mw tool (http://web.expasy.org/compute_pi/). A phylogenetic tree was constructed with MEGA 7.0, and 1000 bootstraps were selected for reliability evaluation [21].

2.5. Quantitative real-time PCR

qRT-PCR was performed to detect the relative expression level of *MrKLF* in LightCycler® 96 (Roche, USA) in accordance with a previous protocol [22]. A pair of gene specific primers for *MrKLF* (MrKLF-qF: 5′-GTCACGCACTCCGAAGAAT-3′; MrKLF-qR: 5′-GAGGTAATGTAGGCACAGCTATAA-3′) were synthesized to amplify a 93 bp DNA target gene fragment. Another pair of specific primers for β-Actin (Mrβ-Actin-qF: 5′-GAGACCTTCAACACCCCAGC-3′, Mrβ-Actin-qR: 5′-TAGGTGGTCTCGTGAATGCC-3′) was also synthesized as the internal reference. The total volume for PCR reaction was 10 μL (5 μL of 2 × TransStart Top Green qPCR SuperMix, 1 μL of 10-fold diluted cDNA, 0.2 μL of 10 mM of each primer, and 3.6 μL of ddH_2O). qRT-PCR was done according to following parameters: 95 °C for 30 s; 40 cycles at 95 °C for 5 s; and 60 °C for 30 s. A melting curve analysis from 60 °C to 95 °C was performed. PCR amplifications were performed in triplicates for each sample. The relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method [23]. A *t*-test was conducted, and statistically significant differences were accepted when $P < 0.05$.

2.6. Interference of MrKLF in vivo by dsRNA injection

Gene specific primers were designed to obtain DNA templates containing a T7 promotor (MrKLF-RNAi-F: 5′-GCGTAATACGACTCACTATAGGAAGCTCGTAG

CAATAGCACCA-3′ and MrKLF-RNAi-R: 5′-GCGTAATACGACTCACTATAGG

CAGCCAAACTTCTTCTCACCC-3′; GFP-RNAi-F: 5′-GCGTAATACGACTCACT

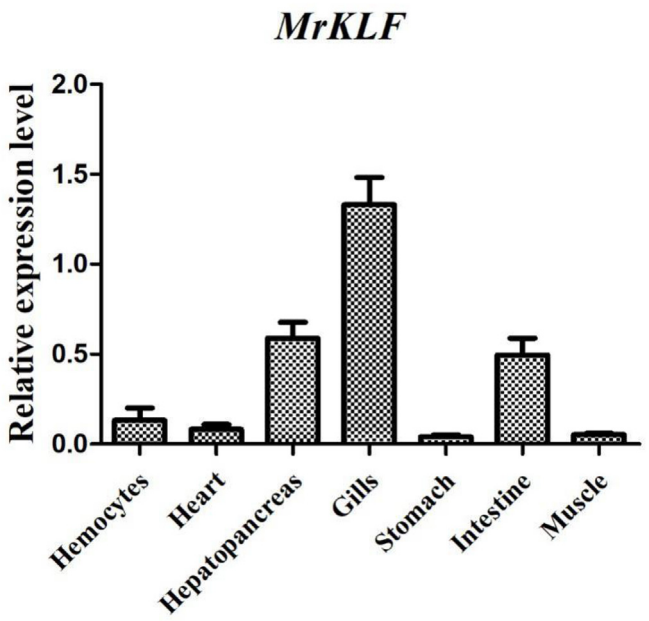

Fig. 4. Tissue distribution of *MrKLF* in hemocytes, heart, hepatopancreas, gills, stomach, intestines, and muscles of the healthy giant freshwater prawn, *M. rosenbergii*.

ATAGGTGGTCCCAATTCTCGTGGAAC-3′ and GFP-RNAi-R: 5′-GCGTAATAC

GACTCACTATAGGCTTGAAGTTGACCTTGATGCC-3′). Double stranded RNA (dsRNA) for *MrKLF* and green fluorescent protein (GFP) were synthesized using T7 RNA polymerase (Fermentas, USA) in accordance with a previously described method [24]. In the RNAi experiment, 30 μg of dsRNA for *MrKLF* or GFP (negative control group) plus *A. hydrophila* (3×10^5 cells) were injected into healthy prawns. After 36 h, gills of five prawns from each group were collected to detect the RNAi efficiency by qRT-PCR. Moreover, total RNA from gills was reversely transcribed into cDNA to determine the relative expression of AMPs, including *MrCrus2*, *MrCrus8*, *MrALF1*, and *MrALF3*. The following primers were used for qRT-PCR analysis: MrCru2-qF: 5′-AATG GCTCGTCTTTGTGTCTT-3′, MrCru2-qR: 5′- CTTTCCACGGGTTGCTTA

GGT-3′; MrCru8-qF: 5′-GTTGGCCATGTTAGTGGGGAC-3′, MrCru8-qR: 5′-CAG

ACAAGGTTTGGGGGAGTT-3′; MrALF1-qF: 5′-ACCAAACAACTCGCAG CAT

AT-3′; MrALF1-qR: 5′-TCCCCTGTCAACCCTCCTAAA-3′; and MrALF3-qF: 5′-GAAGTTGTGGCGGCTGCTGTTG-3′, MrALF3-qR: 5′-CCTGACGA AGTCTTGG

GTTGTT-3′.

3. Results

3.1. Molecular cloning and sequence analysis of MrKLF

A 1799 bp nucleotide sequence representing the complete cDNA sequence of *MrKLF* was acquired. *MrKLF* cDNA contained a 5′ untranslated region (UTR) of 193 bp, a 3′ UTR of 274 bp with a poly(A) tail, and an open reading frame of 1332 bp encoding a polypeptide of 444 amino acids (GenBank ID: MN545962) (Fig. 1). SMART program analysis revealed that predicted MrKLF protein contained three low complexity regions (residues 25–37, 88–100, and 221–230) and three typical ZnF_C2H2 domains (residues 332–356, 362–386, and 392–414). The protein was estimated to have a molecular weight of 48834.74 Da with a pI of 9.33.

3.2. Sequence and phylogenetic tree analysis

The domain organizations of MrKLF with other crustacean KLFs including LvKLF11 (AYM26544.1), LvKLF (AIE48080.1) from *Litopenaeus vannamei*, and PmKLF (AEI83865.1) from *Penaeus monodon* were analyzed in Fig. 2A. All four KLFs contained three ZnF_C2H2 domains, which were conserved across species by multiple alignments analysis (Fig. 2B). BLASTP also showed that MrKLF displayed a relatively moderate identity of 59% with PmKLF, 58% identity with LvKLF, and 51% identity with LvKLF11. The phylogenetic tree of MrKLF was constructed using the neighbor-joining (NJ) method with KLF homologs of invertebrates (Fig. 3). The MrKLF was first clustered with LvKLF11, LvKLF, and PmKLF before being clustered with HaKLF10 (XP_018010554.1) from *Hyalella azteca* and AqKLF10 (XP_003387874.1) from *Amphimedon queenslandica*.

3.3. Tissue distribution of MrKLF

The mRNA expression of *MrKLF* in healthy prawns tissues, including hemocytes, heart, hepatopancreas, gills, stomach, intestine and muscle, was determined using qRT-PCR (Fig. 4). *MrKLF* was expressed in gills, hepatopancreas, and intestines, followed by hemocytes and heart. Relative lower expression levels were obtained in the stomach and muscle.

3.4. Expression profiles of MrKLF after V. parahemolyticus and A. hydrophila challenges

V. parahemolyticus and *A. hydrophila* were used to challenge the prawns, and the temporal mRNA expression of *MrKLF* in gills, intestines, and hepatopancreas were determined by qRT-PCR (Fig. 5). For the *V. parahemolyticus* challenge, *MrKLF* in gills and intestine decreased at 2 h, gradually increased at 6 h, and then reached the peak at 12 h. However, in the hepatopancreas, the *MrKLF* transcript was quickly upregulated at 2 h and then slightly declined. After *A. hydrophila* stimulation in prawns, the highest mRNA level of *MrKLF* was detected at 6 h in the gills, at 12 h in the intestines, and at 24 h in the hepatopancreas. The control group (injected with PBS) showed non-significant variations in *MrKLF* expression level.

3.5. MrKLF regulates the expression of AMPs in vivo

RNAi was carried out to investigate whether *MrKLF* participated in the regulation of expressions of different *AMPs* (Fig. 6). The transcript levels of 13 *AMPs* were determined in prawn gills after the RNAi of *MrKLF*. In the GFP-dsRNA group, at 36 h of injection *A. hydrophila*, the mRNA levels of all *AMPs* were highly induced. When *MrKLF* was knocked down in prawns injected with *MrKLF*-dsRNA plus *A. hydrophila*, at 36 h, a significant downregulation of *MrCrus2*, *MrCrus8*, *MrALF1*, and *MrALF3* was observed in gills. No obvious changes in the transcription levels of *MrCrus1*, *MrCrus3-7*, *MrALF2*, and *MrALF4-5* were found (data not shown). These data suggested that *MrKLF* protects prawns from pathogen invasion by regulating the expression of the four *AMP* genes.

4. Discussion

Invertebrates do not have a real adaptive immunity; they rely solely on their efficient innate immune system to defend themselves against intruding pathogens [25]. The proteins of KLF family are transcription factors that play critical and diverse roles other than in the differentiation and development of various tissues [1,5] and in immune response [26]. Although plenty of circumstantial evidence supports the existence of KLF in invertebrates [26–28], knowledge on the expression

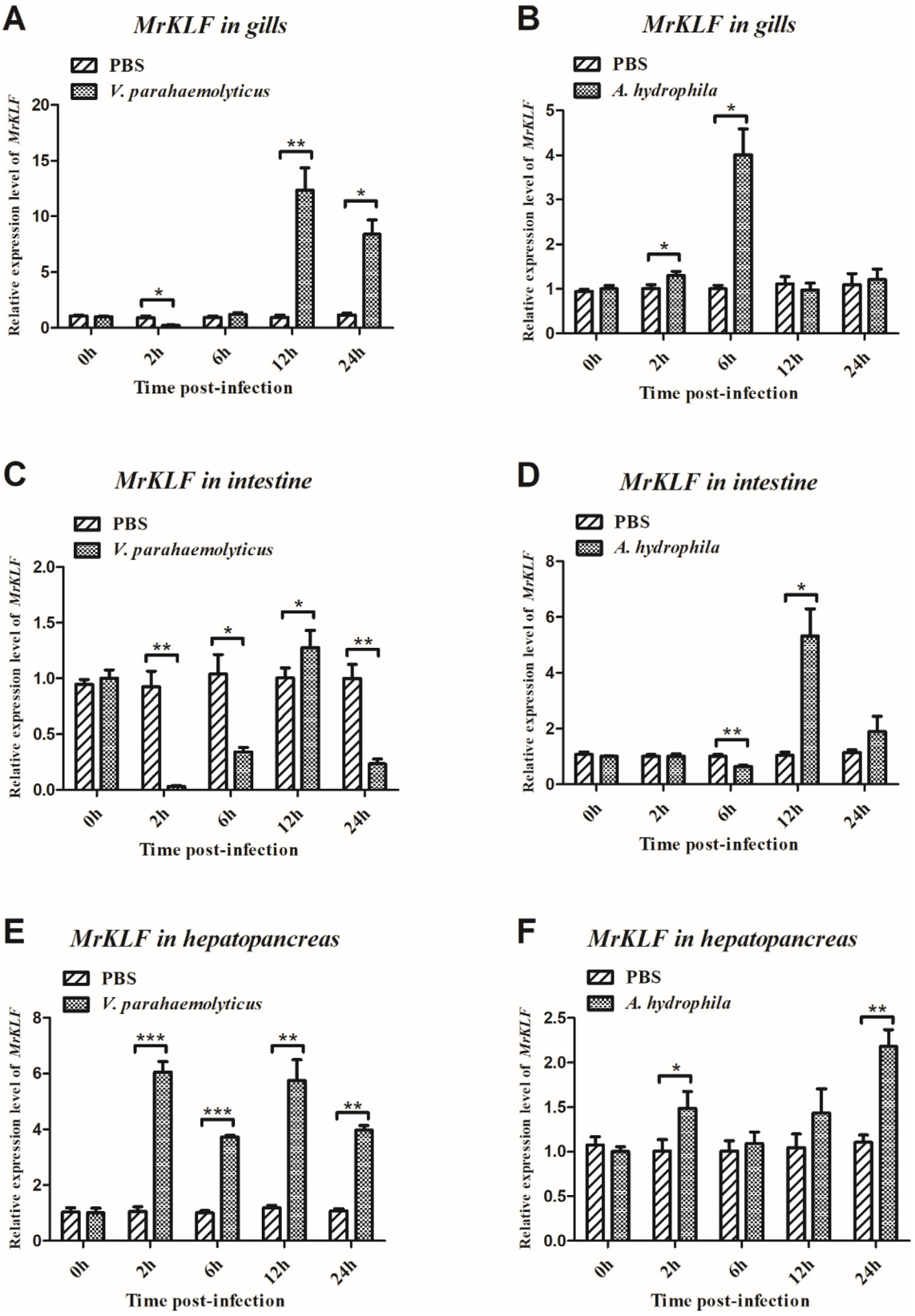

Fig. 5. qRT-PCR analysis of *MrKLF* expression in *M. rosenbergii* gills (A, B), intestine (C, D), and hepatopancreas (E, F) at different time points after *V. parahaemolyticus* and *A. hydrophila* challenge, respectively. Five prawns were chosen to eliminate individual differences at each sampling time point. β-Actin gene was used as the internal control. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$) compared with the values of the PBS group. Error bars represent the mean ± S.D. of three independent experiments.

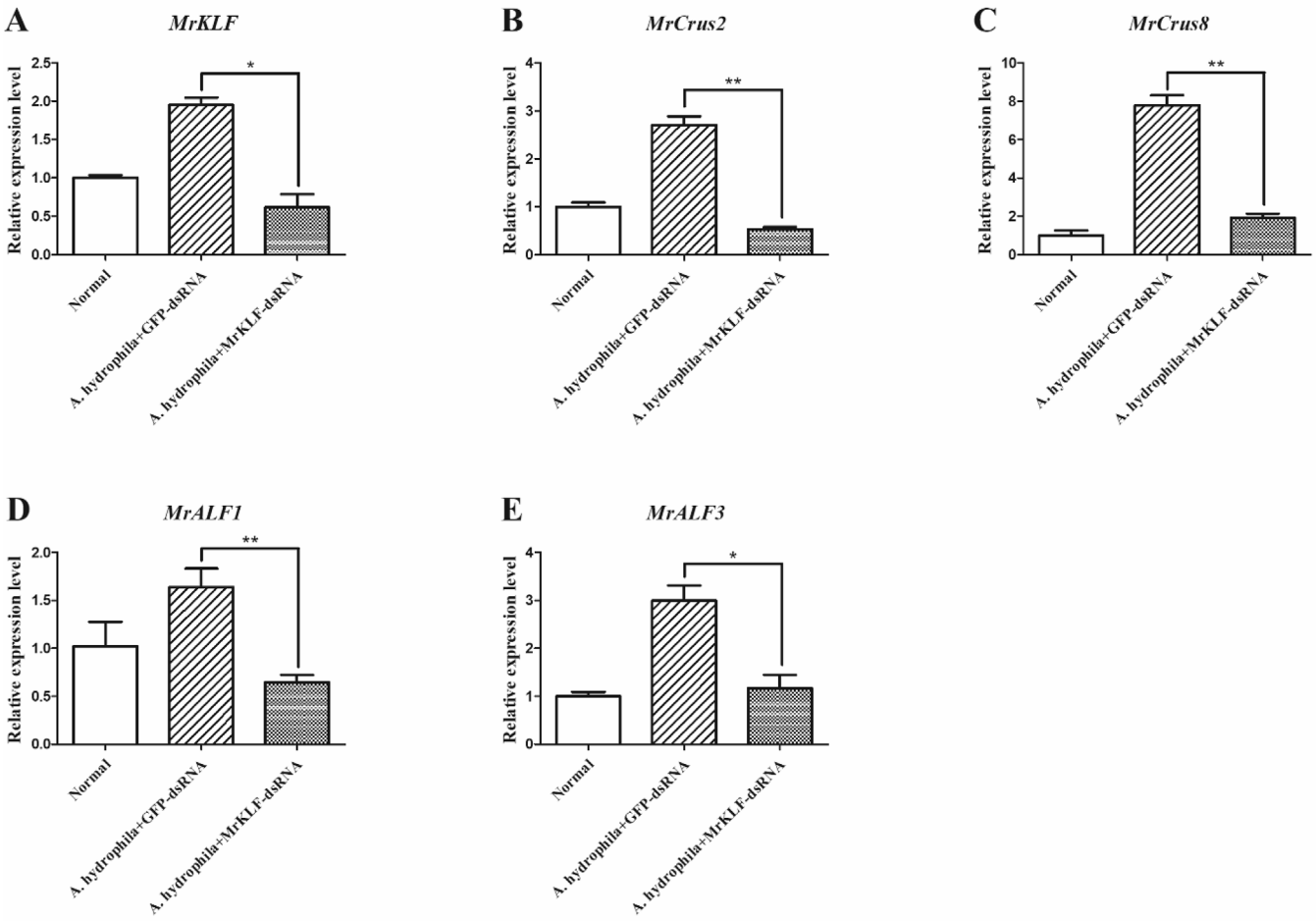

Fig. 6. *MrKLF* regulated the expression of *AMPs in vivo*. (A) RNAi efficiency of *MrKLF*-dsRNA. The mRNA expression level of *MrKLF* was detected in the gills of prawns injected with *MrKLF*-dsRNA or *GFP*-dsRNA plus *A. hydrophila* at 36 h. The mRNA levels of *MrCrus2* (B), *MrCrus8* (C), *MrALF1* (D), and *MrALF3* (E) were measured after RNAi of *MrKLF*. β-Actin gene was used as the internal control. All the data were obtained from three independent experiments and were expressed as mean ± SD. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$).

and regulation of invertebrates KLF is lacking.

In the present study, a novel *MrKLF* of giant river prawn was successfully cloned and characterized. MrKLF consists of 444 amino acids, which is similar to the previously reported KLF protein structures. Sequence analysis indicated that MrKLF contains three conserved ZnF_C2H2 domains located in or near the C-terminal region, which are relatively small protein motifs with evolutionary special functions [1,29,30]. For example, ZnF-containing proteins play crucial roles in gene transcription, translation, mRNA trafficking, protein folding, cell adhesion, and zinc sensing [31]. The high similarity in the ZnF_C2H2 domains of MrKLF suggested that MrKLF might perform similar functions in the prawn immune system, and this idea was worth exploring. MrKLF belongs to the same clade of the crustacean branch as other prawn KLFs and *H. azteca* KLF10, all of which were further separated into different subclades in the NJ phylogenetic tree. The conserved domains and motifs and the high similarity with other KLFs suggested that MrKLF in *M. rosenbergii* is a new member of the KLF family.

As determined by qRT-PCR, the *MrKLF* transcript was found in different tissues of prawn and was detected in the gills, hepatopancreas, and intestines. mRNA expression of other KLFs from crustaceans, such as *PmKLF* [26] and *Pt-Kr-h1* [28], have also been broadly observed in various the tissues tested. *PmKLF* was transcribed in the midgut, muscles, heart, nerves, gills, hepatopancreas, and stomach; transcription levels were relatively lower in gills and hepatopancreas [26]. The transcripts of *Pt-Kr-h1* were most abundant in the muscle of both males and females, followed by Y-organ and hepatopancreas [28]. The gills are frequently exposed to the environment, because they act as filters that are relevant for water and air exchange [32]. As one of the most important organs involved in the innate immune defense of crustaceans, the function of the hepatopancreas in prawn is analogous to that of the fat body in insects [33]. The intestines provide an active environment for a variety of microbes, including pathogens, because of their digestion and absorption functions [34]. Thus, the high expression of *MrKLF* in the gills, hepatopancreas, and intestines of *M. rosenbergii* may be consistent with its roles in host immune defense.

The innate immune system of crustaceans is a complex proteolytic cascade system that may be triggered by the components of pathogens [35]. *Vibriosis* which is a major disease caused by bacteria in the genus *Vibrio*, is causing a serious damage to the aquaculture industry, thereby resulting in heavy economic loss [36]. *A. hydrophila* is a heterotrophic, Gram-negative, and rod-shaped bacterium that is highly toxic to many organisms and is widely considered to be a major pathogen that causes disease outbreaks in *M. rosenbergii* [37]. Therefore, we also used *V. parahaemolyticus* and *A. hydrophila* as immune challengers. When the experimental prawns were exposed to the pathogenic *V. parahemolyticus* and *A. hydrophila*, different expression patterns of *MrKLF* in the gills, intestine, and hepatopancreas were observed. *MrKLF* mRNA in different tissues increased to varying degrees in response to different bacterial infections, thereby indicating the diversity of *MrKLF* functions and that *MrKLF* may plays different roles in immunity under different stimuli.

MrKLF is involved in pathogen-host interactions, thereby suggesting that *MrKLF* may be an immunity-related gene in aquatic animals.

Gene knockdown using dsRNA is a powerful tool for investigating gene function in crustaceans [26,38]. In the giant tiger shrimp *P. monodon*, inhibition of *PmKLF* transcription by dsRNA interference in white spot syndrome virus (WSSV)-infected shrimp resulted in delayed cumulative mortality and reduced the copy number of WSSV and *ie1* expression, thereby revealing that PmKLF affects WSSV infection by interfering with *ie1* expression [26]. Similarity, LvKLF from the pacific white shrimp *L. vannamei* may affect the transcription of WSSV 108 and 140, thereby suggesting that LvKLF is important to WSSV infection [26]. To elucidate the role of MrKLF in antimicrobial infection, dsRNA-mediated knockdown of *MrKLF* transcript was carried out in *M. rosenbergii*. RNAi assay showed that the mRNA levels of four *AMPs* (*MrCrus2*, *MrCrus8*, *MrALF1*, and *MrALF3*) were continuously down-regulated based on the knockdown of *MrKLF* after the *A. hydrophila* challenge. *MrKLF* might have acted as a positive regulator of these four *AMP* genes activation. Crustins, a kind of whey acidic protein domain containing antimicrobial peptides, play important roles in defending against bacteria in the innate immunity system of crustaceans [39,40]. ALFs are effective antimicrobial peptides that can bind and neutralize lipopolysaccharide and is a key effector molecule in the antibacterial immune response of crustaceans [41]. Based on the observations from this study and those in previous studies, *A. hydrophila* entry might induce *MrKLF* activation and subsequently regulate the expressions of *AMP* genes against the invading organisms.

A novel *KLF* gene was identified from *M. rosenbergii* (*MrKLF*). Its mRNA expressions in different tissues and temporal expression in gills, intestine, and hepatopancreas after stimulation with different pathogens were investigated. *MrKLF* was also involved in regulating the expressions of different AMPs *in vivo*. As the first report of KLF homologs in *M. rosenbergii*, the current findings provided information that can be useful in further research on the immune function of KLF in crustaceans.

Author contributions

Y.H. carried out the experiments. Y.H. and Q.R. designed the experiments and analyzed the data. Y.H. contributed reagents/materials. Y.H. and Q.R. wrote the manuscript. All authors gave final approval for publication.

Declaration of competing interest

No potential conflicts of interest were disclosed.

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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.2019.10.070.

Ethics statements

We declare that appropriate ethical approval and licenses were obtained during our research.

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