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RESEARCH REPORT

The first identification of a malectin gene (*CfMal*) in scallop *Chlamys farreri*: sequence features and expression profiles

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Abstract

Malectin is a newly discovered lectin of the endoplasmic reticulum (ER) that might be involved in innate immunity. Information about the roles of malectin in innate immunity is scarce. In the present study, a novel malectin gene (designated as *CfMal*) from the Zhikong scallop *Chlamys farreri* was identified and characterized. Sequence features, tissue distribution, and temporal expression profiles were investigated to infer the potential functions of *CfMal* in innate immunity. The complete cDNA sequence of *CfMal* comprised 1,111 bp and contained an open reading frame of 909 bp, which encoded 302 amino acid residues. A malectin domain and a transmembrane region were identified in the predicted protein sequence. *CfMal* mRNA transcripts were detectable in hemocytes, muscle, mantle, gill, hepatopancreas, and gonads. *CfMal* expression was highest in hemocytes. Stimulation with *Vibrio splendidus* increased *CfMal* expression in hemocytes, gill, and hepatopancreas. The mRNA transcripts of *CfMal* and three related genes, including binding immunoglobulin protein, heat shock protein 90 kDa β member 1 protein and ER degradation enhancing α-mannosidase like protein 1, increased in scallop hemocytes during an artificial ER-stress. Our results indicate that *CfMal* might not only be involved in ER-stress, but may also play a role in innate immunity of scallops.

Key Words: *Chlamys farreri*; innate immunity; malectin

Introduction

Lectins are a large family of evolutionally conserved proteins that bind terminal sugars of glycoproteins or polysaccharides; they act as pattern recognition receptors (PRRs) of the innate immune system (Weis and Drickamer, 1996). Based on carbohydrate ligands, subcellular localization, and dependence on divalent cations, animal lectins could be classified into several groups including C-type, F-type, I-type, L-type, P-type, S-type (also known as galectin), and X-type lectins (also known as intelectins), discoidins, and pentraxins (also known as pentaxins) (Dahms and Hancock, 2002; Kilpatrick, 2002; Arasu *et al*., 2013; Jia *et al*., 2016; Shao *et al*., 2018; Tian *et al*., 2018; Wang *et al*., 2016a; Wei *et al*., 2018).

Lectins localized in the endoplasmic reticulum (ER) are termed as ER-resident lectins (Aebi *et al*., 2010). Most of these ubiquitous lectins participate in host-pathogen interactions and in immunomodulation (Cambi *et al*., 2005). Malectin is a highly conserved membrane-anchored ER-resident lectin; it was first identified in *Xenopus laevis* in 2008 and specifically recognized $Glc_2Man_9GlcNAc_2$ (G_2M_9) in newly synthesized glycoproteins (Schallus *et al*., 2008). Accumulating research shows that malectin is induced by ER-stress and is associated with folding defective glycoproteins to reduce their secretion (Galli *et al*., 2011; Yang *et al*., 2018). What's more, *de novo* characterization of the spleen transcriptome of the large yellow croaker *Pseudosciaena crocea* stimulated with polyinosinic:polycytidylic acid (poly IC) revealed that malectin might be involved in antiviral responses (Mu *et al*., 2014). Moreover,

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three genes, including binding immunoglobulin protein (BiP, also known as 78 kDa glucose regulated protein), heat shock protein 90 kDa β member 1 protein (also known as 94 kDa glucose-regulated protein, Grp94), and ER degradation enhancing α-mannosidase like protein 1 (EDEM1), were previously reported that exhibited close relationship with Mal during ER stress (Galli *et al*., 2011; Qin *et al*., 2012; Merulla *et al*., 2013). In scallops, Grp94 might play an important role in the innate immune defense of the Yesso scallop *Patinopecten yessoensis* (also known as *Mizuhopecten yessoensis*) (Wang *et al*., 2018c). However, the potential roles of malectin in innate immunity are still unclear.

Scallops represent an important aquaculture species with commercial, ecological, and evolutionary importance (Matozzo, 2016; Tascedda and Ottaviani, 2016; Gerdol, 2017; Jielian *et al*., 2017). As invertebrates, scallops lack clonally derived immunoglobulins and T-lymphocytes based adaptive immunity, and depend on their innate immune system to eliminate non-self-particles and to kill invading pathogens (Song *et al*., 2015). In the past two decades, many PRRs have been identified in marine scallops, especially in the bay scallop *Argopecten irradians* and the Zhikong scallop *Chlamys farreri*. These PRRs include C-type lectin (Mu *et al*., 2012), galectin (Song *et al*., 2011), lipopolysaccharide (LPS) and β-1,3-glucan binding proteins (Su *et al*., 2004), leucine-rich repeat-only proteins (Wang *et al*., 2017), peptidoglycan recognition proteins (Ni *et al*., 2007), scavenger receptors (Liu *et al*., 2011), thioester containing proteins (Zhang *et al*., 2007), and Toll-like receptors (Wang *et al*., 2011). These research achievements have enhanced the understanding of the potential functions of these PRRs in invertebrate innate immunity (Song *et al*., 2015).

Therefore, in the current study, we used Zhikong scallops to explore the potential roles of malectin in invertebrate innate immunity. We identified a malectin gene from *C. farreri* (designated as *CfMal*) and we analyzed its expression induced by various stimuli, which indicated its potential role in innate immunity. The main purposes of our present work were: (1) to describe the sequence features of *CfMal*; (2) to investigate the expression profiles of *CfMal*; and (3) to predict the potential functions of *CfMal* in innate immunity.

Materials and Methods

Scallops, in vivo Vibrio *stimulation, and sample collection*

Adult scallops (average 5 cm in shell length) were collected in a local farm in Qingdao, China, in summer; they were maintained in aerated seawater at approximately 20 °C. *Vibrio splendidus* strain JZ6, which has been proved to be a main kind of pathogens for scallop and widely used for the stimulation (Wang *et al*., 2019a; Wang *et al*., 2019b), was cultured in liquid 2216E media (HB0132, HopeBiotech, China) at 28 °C with shaking at 180 rpm overnight. Bacteria were collected by centrifugation at 4000 g for 20 min, and then re-suspended in filtered seawater. Fifteen scallops were immersed for 12 h in filtered water containing live *V. splendidus* at a final concentration of 1.0×10^8 colony forming units per mL at 20 ºC, which constituted the *Vibrio* stimulation group. Hemocytes, muscle, mantle, gill, hepatopancreas and gonads from both infected and control scallops were collected for *CfMal* mRNA expression analysis.

Primary cultured hemocytes, in vitro *ER-stress induction, and sample collection*

Primary cultures of scallop hemocytes were prepared as previously described (each time point has 5 repetitions, and each repetition was a mixture of 3 individuals, Wang *et al*., 2014). Briefly, the hemolymph was withdrawn using a sterile syringe from the adductor muscle and diluted (1:3) in modified anticoagulant Alsever's solution (3.36 $g \cdot L^{-1}$ EDTA, 20.8 $g \cdot L^{-1}$ glucose, 22.5 $g \cdot L^{-1}$ NaCl and 8 $g \cdot L^{-1}$ sodium citrate, pH = 7.0, 1000 mOsm). Approximately 1.0×10^5 scallop hemocyte cells were suspended in 200 μL complete Dulbecco's Modified Eagles Medium (High Glucose, FI101, TransgenBiotech, China) supplemented with 10% *TransSerum* EQ fetal bovine serum (FS201, TransgenBiotech, China), 10% scallop serum, 100 $U \cdot mL^{-1}$ penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin (FG101, TransgenBiotech, China). Cells were added to TC-Treated Multiple Well Plates (24 wells, CLS3527, Corning Costar, USA) and incubated for 12 h at 21 °C in 5% CO_2. Thapsigargin (Tg, T9033, Sigma-Aldrich, USA), tunicamycin (Tun, 654380, Sigma-Aldrich, USA) and LPS (L2630, Sigma-Aldrich, USA) were added to corresponding wells at a final concentration of 300 $ng \cdot mL^{-1}$, 10 $\mu g \cdot mL^{-1}$, and 10 $ng \cdot mL^{-1}$, respectively. These stimuli were considered ER-stress induction groups, according to previous reports (Urano *et al*., 2000; Yoshida *et al*., 2001; Wang *et al*., 2015). Among them, Tg specifically could inhibit the fusion of autophagosomes with lysosomes; the last step in the autophagic process. The inhibition of the autophagic process in turn induces stress on the ER which ultimately leads to cellular death (Ganley *et al*., 2011). Tun is an inhibitor of glycosylation that disturbs protein folding machinery in eukaryotic cells. Tun causes accumulation of unfolded proteins in cell ER and induces ER stress (Namia *et al*., 2016). While the expression of CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), which is an ER stress-induced transcription factor, induces apoptosis. And a previous study demonstrated that LPS-induced CHOP expression does not induce apoptosis, but activates a pro-IL-1β activation process (Nakayama *et al*., 2009). Untreated primary cultures of scallop hemocytes were used as a control. Cells from each experimental group were sampled at 0, 3, 6, 12, 24 and 48 h after stimulation.

RNA isolation, cDNA synthesis, and full-length cDNA cloning

Total RNA was isolated using *TransZol* Up (ET111, TransgenBiotech, China). First-strand cDNA was synthesized using *EasyScript* First-Strand cDNA Synthesis SuperMix (AT301, TransgenBiotech, China) with DNase I (RNase-free, GD201, TransgenBiotech, China). Raw RNA was used as template, and adaptor primer-oligo (dT) as

Table 1 Primers used in the present research

Primer	Sequences (5`-3`)	Brief information
adaptor primer	GGCCACGCGTCGACTAGTAC	Anchor primer for 3` RACE
adaptor primer-oligo (dG)	GGCCACGCGTCGACTAGTACG_{10}HN	Anchor primer for 5` RACE
adaptor primer-oligo (dT)	GGCCACGCGTCGACTAGTACT_{17}VN	Olido (dT) for cDNA synthesizing
*Cf*EF-1α-qRT-F	ATCCTTCCTCCATCTCGTCCT	Internal control for qRT-PCR
*Cf*EF-1α-qRT-R	GGCACAGTTCCAATACCTCCA	Internal control for qRT-PCR
*Cf*Mal-RACE-F1	GCCTCCGATGACACCAGCACC	Gene specific primer for RACE
*Cf*Mal-RACE-F2	CTCTGTAAACTGTAAATATCAGATCAGGGG	Gene specific primer for RACE
*Cf*Mal-RACE-R1	CTTGCATATACACGGCTACACGCCGAC	Gene specific primer for RACE
*Cf*Mal-RACE-R2	GCGAATGTCCAGGAAGTGCGGCTC	Gene specific primer for RACE
*Cf*Mal-CDS-F	ATGGCGCTGCGAGCCGCA	Gene specific primer for CDS
*Cf*Mal-CDS-R	TTACAGTTTACAGAGGCAGAAGAGGAGAGG	Gene specific primer for CDS
*Cf*Mal-qRT-F	AGATTCGCTCAAAGTCGGG	Gene specific primer for qRT-PCR
*Cf*Mal-qRT-R	CGCTGAGTGGGATTTCGT	Gene specific primer for qRT-PCR
*Cf*BiP-qRT-F	GGTCTTCTTCAGGTATCAGCAG	Gene specific primer for qRT-PCR
*Cf*BiP-qRT-R	CTTATCTTCCTCAGCAAACATTTCC	Gene specific primer for qRT-PCR
*Cf*Grp94-qRT-F	TCCCAGACGACGAACTTAATCCA	Gene specific primer for qRT-PCR
*Cf*Grp94-qRT-R	GTTACCCATTATTGCCAGAGTGTCC	Gene specific primer for qRT-PCR
*Cf*EDEM1-qRT-F	AGCACCAGTTAAGGATTCTAATGTT	Gene specific primer for qRT-PCR
*Cf*EDEM1-qRT-R	CCACTTCCTCCATACGACTTG	Gene specific primer for qRT-PCR
M13-47	CGCCAGGGTTTTCCCAGTCACGAC	Vector primer for sequencing
RV-M	GAGCGGATAACAATTTCACACAGG	Vector primer for sequencing

*The efficiency of *Cf*EF-1α-qRT-F/R, *Cf*Mal -qRT-F/R, *Cf*BiP-qRT-F/R, *Cf*Grp94-qRT-F/R and *Cf*EDEM1-qRT-F/R were 98%, 103%, 101%, 97% and 99%, respectively

primer (Table 1), according to the manufacturer instructions. Subsequently, a homopolymeric tail was added using terminal deoxynucleotidyl transferase (EP0161, ThermoFisher, USA) and dCTP (10217016, ThermoFisher, USA). We previously identified a malectin homologue sequence of *C. farreri* by using available public transcriptomic data (Wang *et al*., 2018b). And it was selected to clone the full-length cDNA sequence of *CfMal*. Gene-specific primers, *CfMal*-RACE-R1/2 and *CfMal*-RACE-F1/2 (Table 1), were designed using Primer Premier 5.00 to obtain the full-length cDNA sequence of *CfMal* using the rapid-amplification of cDNA ends (RACE) method. All PCR reactions were performed in an MJ Mini Personal Thermal Cycler (Bio-Rad, USA). The PCR products were directly ligated into the *pEASY*-T3 Cloning Vector (CT301, ThermoFisher, USA). After transformation into phage resistant chemically competent cell *Escherichia coli* strain *Trans1-T1* (CD501, TransgenBiotech, China), positive recombinants were selected using *TransCult* LB Agar Plates (Ampicillin, CP111, TransgenBiotech, China) and verified by PCR screening with vector primers M13-47 and RV-M (Table 1). Three positive clones were sequenced in a 3730XL automated sequencer (ThermoFisher, USA) by Genscript Biotech (Nanjing) Inc.

Bioinformatics analysis of cDNA and protein sequences

The protein sequence of *CfMal* was deduced and analyzed by the EditSeq module of Lasergene 7.1.0.44. *CfMal* and the three related genes were identified from the genome of *C. farreri* using BLAST+ 2.8.0, as described previously (Galli *et al*., 2011; Wang *et al*., 2007). The protein sequence similarity search was also conducted by BLAST+ 2.8.0. The presence and location of signal peptides and functional domains were predicted using SignalP 4.1 and Simple Modular Architecture Research Tool (SMART) 8.0. A phylogenetic tree was generated with MEGA-X 10.0.1 using the Neighbor-Joining (NJ) method. Bootstrap trials were replicated 1,000 times to derive a confidence value for phylogenetic analysis.

Analysis of mRNA expression patterns via quantitative real-time PCR (qRT-PCR)

The expression of *CfMal* was analysed by qPCR in several tissue form control and *Vibrio* infected animals as well as in hemocytes during induced ER-stress. All qRT-PCR reactions were carried out using *TransStart* Green qPCR SuperMix UDG (AQ111, TransgenBiotech, China) in a LineGene K FQD-48A Fluorescence Quantitative PCR Detection System (Bioer, China). All the primers

A

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   1                                                  M  A  L  R  A  A  L  P  G  H  S  P  V  W  T  S  S
   1 GGGGTTCGCTCCCTCCTTGTGCCTCCACAGGATTTAGGAGATGGCGCTGCGAGCCGCACTTCCTGGACATTCGCCGGTTTGGACAAGTAG
  18  Q  N  M  L  S  A  C  S  R  V  Y  A  S  M  T  F  C  F  F  H  R  K  H  M  L  F  L  I  C  T
  91 TCAAAACATGTTGTCGGCGTGTAGCCGTGTATATGCAAGCATGACCTTCTGCTTTTTCCACAGGAAGCACATGCTTTTCCTTATATGCAC
  48  I  L  S  L  V  T  Q  S  L  G  I  G  E  V  I  W  A  V  N  C  G  G  E  S  H  T  D  I  N  G
 181 AATTTTGTCCTTAGTAACGCAATCCCTAGGAATTGGTGAGGTTATCTGGGCCGTGAACTGTGGAGGAGAATCCCACACCGATATCAATGG
  78  I  R  Y  E  T  D  S  L  K  V  G  I  S  S  D  Y  G  K  T  L  M  V  S  R  V  V  A  Q  D  Q
 271 TATCCGGTATGAAACAGATTCGCTCAAAGTCGGGATCTCATCAGACTATGGAAAAACATTAATGGTTTCCCGGGTGGTAGCTCAGGACCA
 108  I  L  Y  Q  T  E  R  Y  H  M  S  T  F  G  Y  E  I  P  L  S  G  D  G  E  Y  V  L  V  L  K
 361 GATCTTGTACCAAACTGAGCGATATCACATGTCAACGTTTGGATACGAAATCCCACTCAGCGGAGACGGTGAATATGTCCTCGTCCTCAA
 138  F  C  E  V  W  F  T  S  P  N  Q  K  V  F  D  V  T  L  N  G  E  H  T  V  V  D  E  L  D  I
 451 ATTCTGTGAAGTTTGGTTCACATCACCGAATCAAAAAGTATTTGATGTCACGTTGAATGGAGAACACACTGTTGTGGATGAATTGGATAT
 168  Y  S  K  V  G  R  G  V  A  H  D  E  L  I  E  F  T  I  R  S  G  K  L  K  V  N  G  E  T  S
 541 TTACAGTAAAGTCGGACGAGGAGTGGCACATGACGAACTTATAGAGTTCACAATTAGATCAGGGAAACTCAAAGTGAATGGCGAAACATC
 198  K  I  N  S  K  L  L  V  E  F  M  K  G  D  Y  D  N  P  K  I  N  A  I  Y  L  M  K  G  T  I
 631 AAAAATCAACAGCAAACTTTTAGTAGAATTTATGAAGGGAGATTATGATAATCCGAAAATTAATGCAATATATTTAATGAAGGGAACAAT
 228  D  D  V  P  K  L  P  S  L  P  G  T  E  T  T  R  E  E  E  E  V  D  E  E  E  D  S  P  D  R
 721 AGACGATGTACCCAAGTTGCCATCATTACCAGGGACAGAGACGACAAGAGAAGAGGAAGAGGTAGATGAAGAGGAAGACTCTCCTGATCG
 258  P  S  K  A  R  R  P  S  G  P  K  V  K  D  P  Y  A  S  D  D  T  S  T  M  L  L  P  V  I  I
 811 TCCATCAAAGGCACGTCGACCTTCAGGTCCCAAGGTCAAGGACCCTTACGCCTCCGATGACACCAGCACCATGCTTCTACCCGTTATCAT
 288  A  L  G  A  F  I  P  L  L  F  C  L  C  K  L  *
 901 TGCCCTGGGAGCTTTCATTCCTCTCCTCTTCTGCCTCTGTAAACTGTAAATATCAGATCAGGGGTTCATATGTGTAAGGTCAAAGTTGAA
 991 CATGTGTAAGTTCAAAGTTAAACATGTGTAAGGTCAAAGTTGAACATGTGTAAGTTCAATAAACATGTGTAAGGTCAAAGTTGAACAAAA
1081 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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B

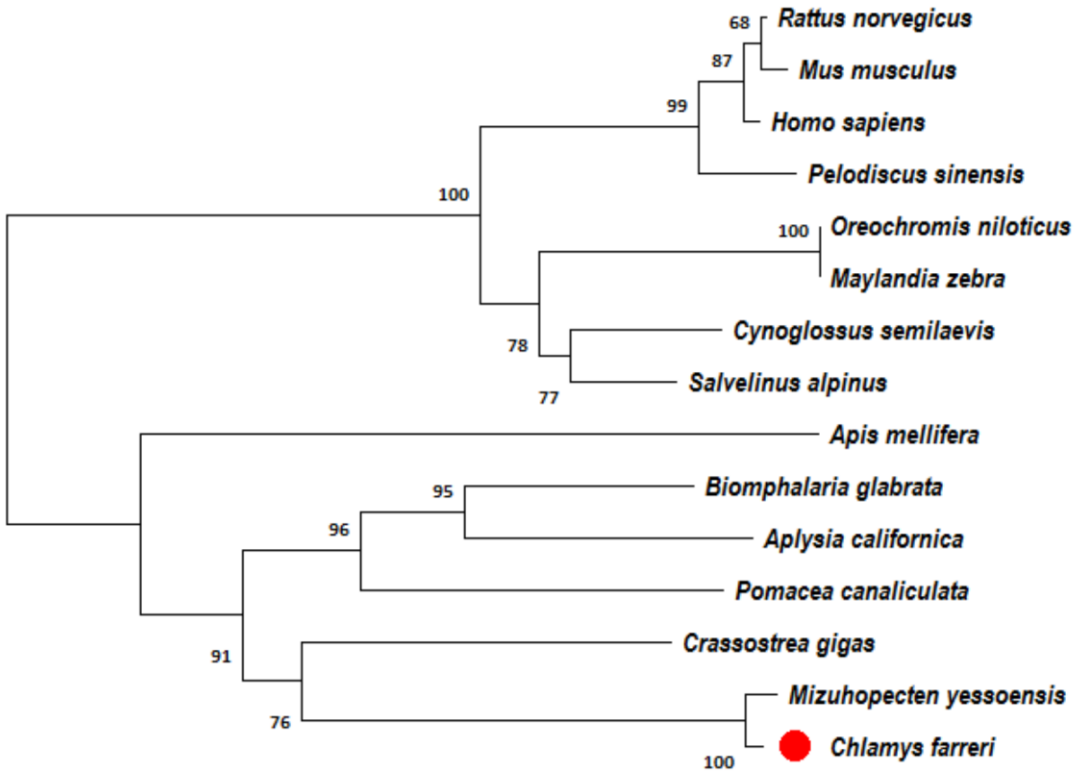

Fig. 1 Sequence features and phylogenetic relationships of *CfMal*. A. Nucleotide and predicted protein sequences of *CfMal*. The nucleotides and amino acids are numbered on the left margin. The function domain is shaded. The low complexity is boxed. The transmembrane region has a double underline. The stop codon is indicated by asterisks. The polyadenylation signal site (AATAAA) is underlined. B. Phylogenetic tree based on the protein sequences of different malectins. The NJ model was used to infer the evolutionary history. The numbers at the branches indicate the bootstrap value (%). The accession numbers of these sequences are as follows: *Apis mellifera*, XP_006563359; *Aplysia californica*, XP_005104301; *Biomphalaria glabrata*, XP_013067932; *Chlamys farreri*, AYB71126; *Crassostrea gigas*, XP_011422439; *Cynoglossus semilaevis*, XP_016898532; *Homo sapiens*, NP_055545; *Maylandia zebra*, XP_004558571; *Mizuhopecten yessoensis*, XP_021354488; *Mus musculus*, NP_780612; *Oreochromis niloticus*, XP_005473062; *Pelodiscus sinensis*, XP_006114823; *Pomacea canaliculata*, XP_025090097; *Rattus norvegicus*, NP_001014005 and *Salvelinus alpinus*, XP_023857162

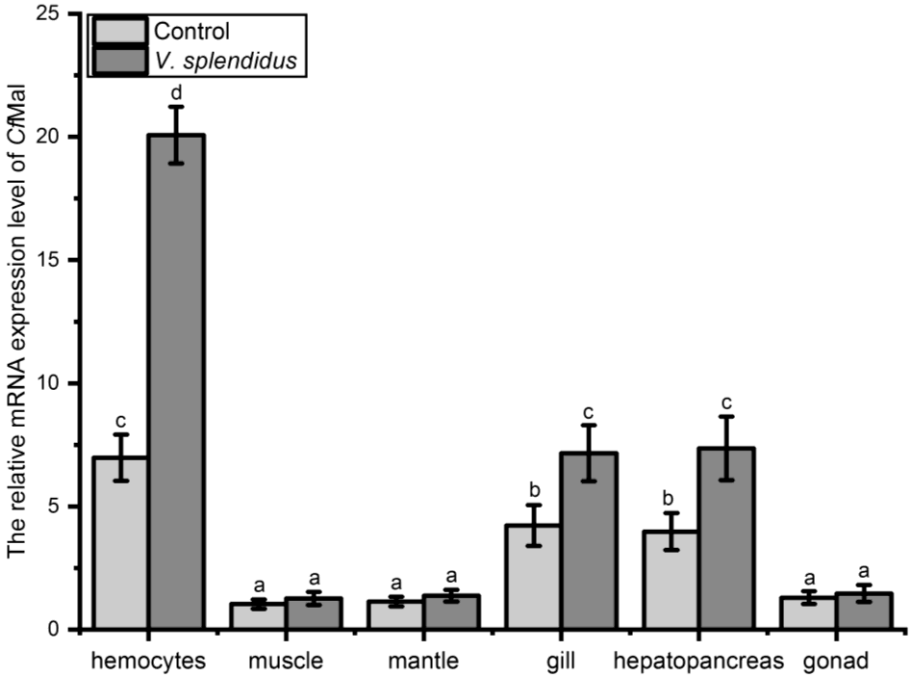

Fig. 2 Spatial mRNA expression patterns of *CfMal*. mRNA expression levels in hemocytes, mantle, gill, hepatopancreas, and gonads of five adult scallops were normalized to that of muscle. Vertical bars represent mean ± SD (n = 5); different letters represent statically significant differences ($p < 0.05$)

for qRT-PCR were designed with PerlPrimer 1.1.21 (Table 1). The threshold cycle (C_T) slope method, based on serial two-fold dilutions of cDNA, was used to confirm that all pairs of these primers had similar efficiency (Pfaffl *et al*., 2001; Wang *et al*., 2018a). For each sample, the expression level of target genes was normalized to that of elongation factor 1 α (*CfEF*-1α). The relative mRNA abundance of target genes was determined using the comparative C_T ($2^{-\Delta\Delta Ct}$) method (Schefe *et al*., 2006; Schmittgen and Livak, 2008). Data are presented as mean ± SD (n = 5, each time point has 5 repetitions, and each repetition was a mixture of 3 individuals); data was subjected to one-way analysis of variance, followed by a multiple comparison using IBM SPSS Statistics software 25.0.0.0. $p < 0.05$ was considered as statistically significant.

Results

Molecular features of CfMal *and its phylogenetic relationship*

The full-length cDNA sequence of *CfMal* obtained via RACE was submitted to GenBank under the accession number MG546685. The complete cDNA sequence of *CfMal* was 1,111 bp long and consisted of a 40 bp 5' untranslated region (UTR), a 3' UTR of 162 bp, and an open reading frame (ORF) of 909 bp. A polyadenylation signal site (AATAAA) was revealed upstream of the polyA tail. The ORF encoded 302 amino acid residues with a predicted molecular mass of 33.665 kDa, and an isoelectric point of 5.210. A malectin domain (from V^{61} to I^{220}) and a transmembrane region (from T^{278} to C^{300}) were identified in the predicted protein sequence by SMART analysis; no signal peptide was revealed (Figure 1A). BLAST+ search revealed that *CfMal* shared high identity with its homologues from *M. yessoensis* (97% identity), *Crassostrea virginica* (75% identity) and *Pomacea canaliculata* (68% identity). Phylogenetic analysis showed that *CfMal* clustered with its counterparts from *M. yessoensis* and formed a sister branch to their homologue from *Crassostrea gigas* (Figure 1B).

Tissue distribution of CfMal *mRNA transcripts*

The tissue distribution of *CfMal* mRNA transcripts was detected by qRT-PCR using *CfEF-1α* as an internal control. *CfMal* mRNA transcripts were detectable in all the sampled tissues; the highest expression was that of hemocytes, which was 6.98-fold ($p < 0.05$, relative to muscle), followed by gill (4.23-fold, $p < 0.05$) and hepatopancreas (3.98-fold, $p < 0.05$). After stimulation with *Vibrio* for 12 h, *CfMal* expression increased significantly in hemocytes, hepatopancreas, and gill (20.07-, 7.36-, and 7.16-fold, respectively, relative to muscle with no stimulation, $p < 0.05$). No significant differences were observed in muscle, mantle, or gonads, before and after stimulation with *Vibrio* (Figure 2).

Temporal expression of CfMal *and related genes during ER-stress induction*

CfMal and the three related genes (*CfBiP*, *CfGrp94* and *CfEDEM1*) were identified from the genome of *C. farreri* using BLAST+ 2.8.0, according to previous description (Wang *et al*., 2007; Galli *et al*., 2011). The expression patterns of these genes were

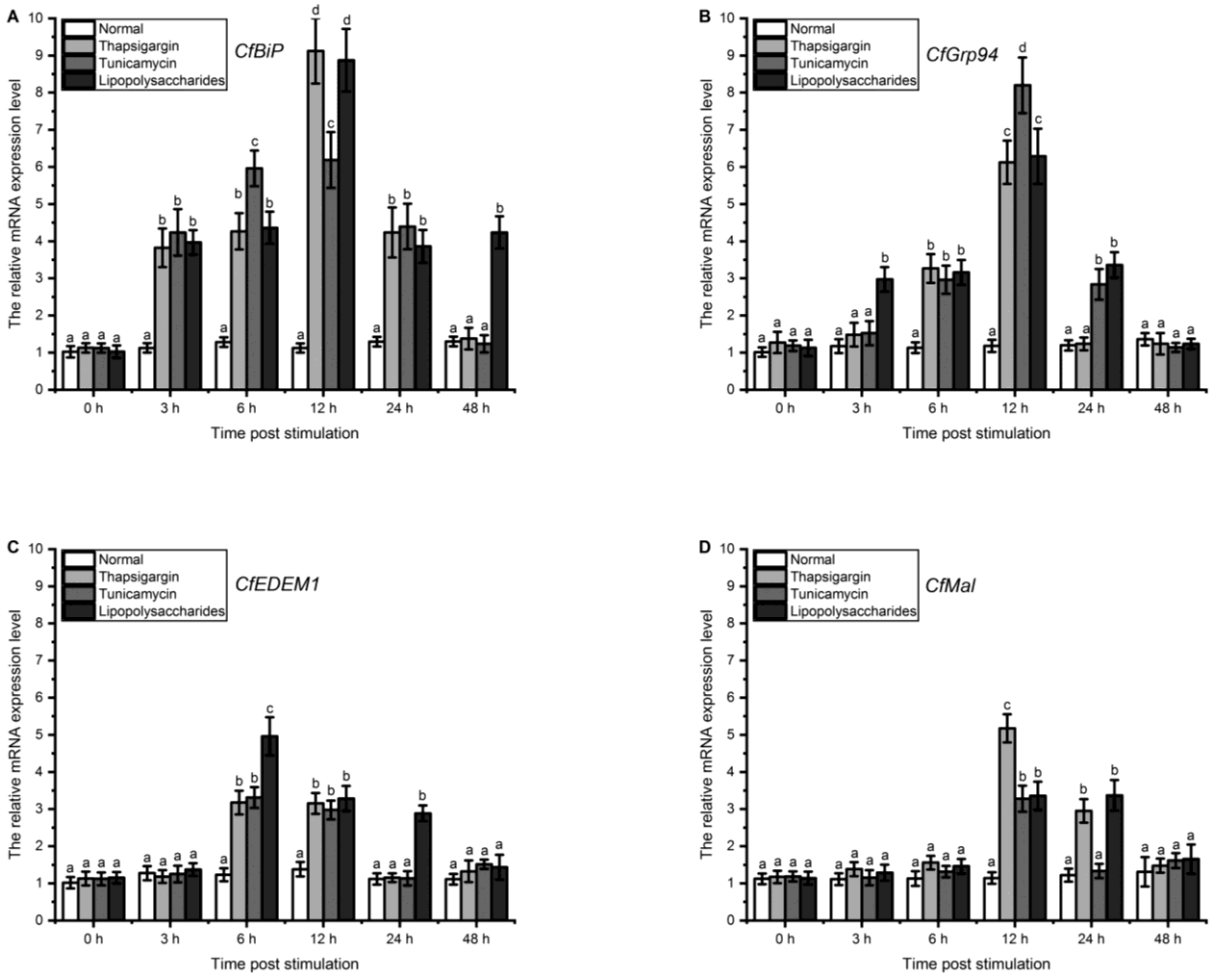

Fig. 3 Temporal mRNA expression patterns of *CfMal* and *CfMal*-related genes during ER-stress. Vertical bars represent mean ± SD (n = 5); different letters represent statically significant differences ($p < 0.05$). A. *CfBiP* B. *Cf*Grp94 C. *CfEDEM1* D. *CfMal*

analyzed by qRT-PCR. The expression of these four genes all increased after hemocytes were stimulated with Tg, Tun, or LPS.

After 3 h of Tg stimulation, the expression of *CfBiP* increased significantly (3.82-fold, $p < 0.05$), reached a peak at 12 h (9.12-fold, $p < 0.05$), and returned to basal levels after 48 h. After 3 h of Tun stimulation, the expression of *CfBiP* increased significantly (4.24-fold, $p < 0.05$), reached a peak at a 6 and 12 h (5.96-fold and 6.19-fold, $p < 0.05$, respectively), and returned to basal levels after 48 h. After 3 h of LPS stimulation, the expression of *CfBiP* increased significantly (3.97-fold, $p < 0.05$), reached a peak at 12 h (8.87-fold, $p < 0.05$), and decreased at 24 and 48 h after stimulation (3.86- and 4.24-fold, respectively, $p < 0.05$ for both; Figure 3A).

After 6 h of Tg stimulation, the expression of *CfGrp94* increased significantly (3.27-fold, $p < 0.05$), reached a peak at 12 h (6.12-fold, $p < 0.05$), and returned to basal levels after 24 h. After 6 h of Tun stimulation, the expression of *CfGrp94* increased significantly (2.96-fold, $p < 0.05$), reached a peak at 12 h (8.20-fold, $p < 0.05$), and returned to basal levels after 24 h. After 3 h of LPS stimulation, the expression of *CfGrp94* increased significantly (2.97-fold, $p < 0.05$), reached a peak at 12 h (6.29-fold, $p < 0.05$), and returned to basal levels after 48 h (Figure 3B).

Expression of *CfEDEM1* peaked after 6 h of Tg stimulation (3.17-fold, $p < 0.05$), and returned to basal levels after 12 h. Expression of *CfEDEM1* peaked after 6 h of Tun stimulation (3.31-fold, $p < 0.05$), and returned to basal levels after 12 h. Expression of *CfEDEM1* peaked after 6 h of LPS stimulation (4.96-fold, $p < 0.05$), and gradually returned to basal levels after 48 h (Figure 3C).

Expression of *CfMal* peaked after 12 h of Tg stimulation (5.17-fold, $p < 0.05$), decreased significantly after 24 h (2.95-fold, $p < 0.05$), and returned to basal levels after 48 h. Expression of *CfMal* increased only after 12 h of Tun stimulation (3.28-fold, $p < 0.05$). Expression of *CfMal* peaked after 12 h of LPS stimulation (3.36-fold, $p < 0.05$) and returned to basal levels after 48 h (Figure 3D).

Discussion

Malectin is a newly discovered ER-resident lectin, which specifically recognizes G_2M_9 in newly synthesized glycoproteins (Schallus *et al*., 2008). Recent research indicates that malectin might play potential roles in innate immunity (Mu *et al*., 2014; Wang *et al*., 2018c). However, information about the role of malectin in innate immunity is scarce. In the present study, we identified a novel malectin gene (*CfMal*) in Zhikong scallop *C. farreri*. We analyzed *CfMal* sequence features, its tissue distribution, and temporal expression profiles, in order to predict its potential functions in innate immunity.

Bioinformatics analysis revealed that *CfMal* contained a typical malectin domain, and exhibited high identity with its invertebrate counterparts. Additionally, in the NJ phylogenetic tree *CfMal* clustered with its homologues from *M. yessoensis* and *C. gigas*. The conserved function domain has high similarity with that of other invertebrates. These phylogenetic relationships suggest that *CfMal* belongs to the invertebrate malectin family.

To investigate the potential functions of *CfMal* in scallops, the tissue distribution of its mRNA transcripts was analyzed. *CfMal* mRNA transcripts could be detectable in all the sampled tissues; expression was highest in hemocytes, followed by gill and hepatopancreas. Hemocytes play pivotal functions in invertebrate innate immunity (Jia *et al*., 2017; Jia *et al*., 2018). Gill is a potential hematopoietic position in mollusks and is the first line of defense against invading microbes in lower animals (Li *et al*., 2017). The hepatopancreas is considered as main immune organ in crustaceans and mollusks (Wang *et al*., 2016b). The high abundance of *CfMal* mRNA transcripts in these tissues indicates that it might be involved in the innate immunity of scallops. *CfMal* expression in these tissues was significantly up-regulated by *Vibrio* stimulation, especially in hemocytes, which confirmed this hypothesis.

To further investigate the potential roles of *CfMal* in scallops, the temporal expression profiles of *CfMal* and three related genes was investigated in hemocytes stimulated with Tg, Tun, or LPS. In previous report, the expression of mammalian BiP, Grp94, EDEM1 and malectin is up-regulated during Tg-induced ER-stress (Galli *et al*., 2011). In the present study, the expression of *CfBiP*, *Cf*Grp94, *CfEDEM1* and *CfMal* increased in scallop hemocytes after stimulation with either Tg, Tun, or LPS. Tg and Tun induced similar gene expression modification in hemocytes, which confirmed the hypothesis that *CfMal* might play a role in ER-stress of scallops. While LPS treated hemocytes showed slightly differences compared to the other stimuli, indicating *CfMal* might also be involved in innate immunity of scallops.

In conclusion, a novel malectin gene (*CfMal*) was identified and characterized in *C. farreri*, including sequence features and expression profiles. The expression of *CfBiP*, *Cf*Grp94, *CfEDEM1* and *CfMal* increased in scallop hemocytes after stimulation with either Tg, Tun, or LPS. The present study provides useful information about the potential functions of *CfMal* in scallops.

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