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RESEARCH REPORT

Sequence feature and expression profile of a tumor suppressor QM protein gene from hard clam *Meretrix meretrix*

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Abstract

The present study describes the molecular characterization and transcriptional features of a tumor suppressor QM protein gene, *MmQM*, in *Meretrix meretrix*. The full-length cDNA (819 bp) of *MmQM* consists of a 657 bp opening reading frame (ORF) encoding a 218 amino acid protein with a calculated molecular mass of 25.3 kDa and theoretical isoelectric point of 10.2. A ribosomal protein L10 signature, an SH_3-binding motif, an antibiotic binding site, an amidation site and two protein kinase C phosphorylation sites were revealed from the *MmQM* sequence. Phylogenetic analysis showed that *MmQM* is clustered with previously identified mollusk QM proteins. *MmQM* mRNA transcripts were detectable in all of the examined tissues in a constitutive manner, and were significantly different from each other. After bacterial stimulation, the mRNA transcripts of *MmQM* in the hepatopancreas significantly increased. Hence, we conclude that *MmQM* could respond to pathogenic infections and it might play an important role in the innate immunity against microorganisms in the clam *M. meretrix*.

Key Words: Innate immunity; *Meretrix meretrix*; QM protein; *Vibrio splendidus*

Introduction

The clam *Meretrix meretrix* is a crucial commercial marine bivalve in coastal and estuarine areas of south and southeast Asia. The rapid development of *M. meretrix* farming depends on its high economic value (Liu *et al*., 2006; Tang *et al*., 2006; Li *et al*., 2011). Recently, the aquaculture of *M. meretrix* has suffered from diseases, and many of these diseases are provoked by Gram-negative bacteria (Yue *et al*., 2010). Especially, *Vibrio* has been reported to be the main pathogenic bacteria causing extensive mortality of *M. meretrix* (Wang *et al*., 2011). Therefore, it is necessary to understand the innate immune defense mechanisms against *Vibrio* of *M. meretrix* to provide new insights into health management and disease control in molluscan aquaculture (Yang *et al*., 2011).

Many previous studies have focused on this field and have discovered several genes engaged in the immune response of *M. meretrix*, including serum amyloid A (Zou and Liu, 2015), γ-aminobutyrate type A receptor-associated protein (GABARAP) (Zhang *et al*., 2014), heat shock protein (Yue *et al*., 2011), IκB protein (Yang *et al*., 2011), cysteine-rich intestinal protein (Chen *et al*., 2014), macrophage migration inhibitory factor (MIF) (Zou and Liu, 2016) and tetraspanins (Wang *et al*., 2019).

Accumulating evidence has demonstrated that the QM gene is associated with tumor suppression and is engaged in the innate immune responses. In humans (Inada *et al*., 1997; Han *et al*., 2015), mammals (Inada *et al*., 1997), fish (Han *et al*., 2015), insects (Zhou *et al*., 2019), arthropods (Zhou *et al*., 2011), yeast (Koller *et al*., 1996), and even fungi (Wen *et al*., 2005) and plants (Bhardwaj *et al*., 2010) the QM gene has been reported to respond to viral and bacterial infection. The sequence of QM is highly conserved, which further implies that QM has stabilized and significant functions across species (Koller *et al*., 1996; Inada *et al*., 1997; Wen *et al*., 2005; Xu *et al*., 2008; Bhardwaj *et al*., 2010; Oh *et al*., 2010; Zhou *et al*., 2011; Chen *et al*., 2015; Han *et al*., 2015).

In particular, recent studies have suggested that QM could facilitate immune response in mollusks, and that the expression level of *AbQM* from *Haliotis discus* could be significantly upregulated in gills

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upon bacterial (*Vibrio alginolyticus*, *Vibrio parahemolyticus* and *Listeria monocytogenes*) and viral (hemorrhagic septicemia virus) challenge, suggesting it regulates the defensive effect against pathogenic infections (Oh *et al*., 2010). Subsequently, *CfQM* protein from *Chlamys farreri* was associated with its host defense against bacterial (*Vibrio anguillarum*) and viral (acute viral necrobiotic virus) infections (Chen *et al*., 2015). Moreover, several studies have revealed that the QM proteins are involved in cell growth, differentiation and apoptosis (Marty *et al*., 1993; Lillico *et al*., 2002; Zhang *et al*., 2004), suggesting this gene is associated closely with cell viability.

The current study reports a QM gene (*MmQM*) from *M. meretrix*. The full-length cDNA of *MmQM* was obtained via a rapid amplification of cDNA ends (RACE) technique. Then, the tissue-specific expression of the *MmQM* gene was investigated. Finally, the immune response of the gene to bacterial challenges was analyzed.

Materials and methods

Experimental clams, immune challenge and sample collection

A total of 300 healthy clams (*M. meretrix*) with an average shell height of 2 cm were purchased from an aquatic market in Qingdao, China. The clams were maintained at approximately 21 °C for one week before processing. Approximately 210 clams were employed for the microbe stimulation assay. *Vibrio splendidus* strain JZ6 was employed as the pathogen (Liu *et al*., 2013). One hundred and five clams were chosen randomly to be kept in tanks containing live *V. splendidus* (10^8 CFU mL^{-1}) suspended in seawater based on the findings of a previous report (Wang *et al*., 2018) while the other clams were cultivated in seawater as the control group. After stimulation, hepatopancreas samples were randomly selected from both the challenged and control groups at 0, 3h, 6h, 12h, 24h, 48h and 96h, with 5 replications at each time point, and each replication was a mixture of 3 individuals. Six kinds of tissues from unstimulated clams, including hemocytes, hepatopancreas, mantle, foot, gill and adductor muscle, were dissected and stored at −80 °C to analyze the tissue transcripts distribution of *MmQM*. Particularly, the hemocytes sample was prepared by collecting the hemocyte pellet after centrifugation of hemolymph from three clams for 10 min at 800 *g* at 4 °C. One analytical sample consisted of three individual clams. Five replicates were performed for each type of tissue.

Isolation of total RNA and synthesis of cDNA

The total RNA was isolated using TRIzol Reagent (ThermoFisher, USA) according to the manufacturer's protocol. The purified RNA was precipitated and dried for 5 min, and then dissolved in 10 μL of DEPC-treated water. Before cDNA synthesis, 4 μL of RNA was used to identify its quality by electrophoresis; the RNA concentration was determined by measuring the absorbance at 260 nm in a Nanodrop 2000c. The 10 μL cDNA synthesis mixture containing 6 μL of RNA (1 μg), 1 μL of RQ1 Buffer, 2 μL of RQ1 DNaseI (Promega, USA) and 1 μL of RNase Inhibitor (Promega, USA) was incubated at 37 °C for 30 min. Inactivation was performed at 65 °C for 10 min with 1 μL of RQ1 Stop solution before 1 μL of 50 mM Oligo-dT was added, then heat denaturation was performed at 70 °C for 5 min. The reaction was terminated by cooling on ice for 2 min, and then centrifuged briefly. Then, a mixture containing 5 μL of FS Buffer, 1 μL of dithiothreitol (DTT, 0.1 M), 1 μL of RNase Inhibitor, 5 μL of 2.5 mM dNTPs and 1 μL of SuperScript III (ThermoFisher, USA) was briefly centrifuged. The reverse transcription was conducted for 1h at 55 °C and inactivation was performed at 70 °C for 15 min.

Molecular cloning of the MmQM *cDNA*

Based on a partial cDNA sequence (GR902610) of a homologue to the QM gene in clam *M. meretrix* obtained in a previous study (Li *et al*., 2011), a pair of sequence-specific primers F1 and F2 (Table 1) were designed using Primer Premier 5.0 and used for PCR amplification to clone the full-length cDNA of *MmQM*. The PCR products were gel-purified and cloned into the pMD-19T simple vector (Takara, Japan). After being transformed into competent cells of *Escherichia coli* strain Top 10 (Tiangen, China), the potentially positive recombinant clones were identified through anti-ampicillin selection and verified by PCR screening. The sequencing was carried out using an ABI 3730 sequencer (ThermoFisher, USA).

Table 1 Information of primers used in this study

Primer	Length (bp)	Sequence	Tm(°C)
F1	27	5'-GAGCCAAGTTCAAGTTCCCAGGCAGAC-3'	71.75
F2	27	5'-ATCCCAGATGGTGTCAGTGTACAGTAC-3'	68.54
QM-F	20	5'-CGAGCCAAGTTCAAGTTCCC-3'	64.05
QM-R	24	5'-CTGGGATAAGCTGATGTTTCTGTC-3'	64.02
β-actin-F	21	5'-TTGTCTGGTGGTTCAACTATG-3'	60.99
β-actin-R	20	5'-TCCACATCTGCTGGAAGGTG-3'	64.74

Sequence characterization and phylogenetic analysis of MmQM

The cDNA sequence of *MmQM* was analyzed using BLAST (www.ncbi.nlm.nih.gov/blast) and ORF Finder (www.ncbi.nlm.nih.gov/projects/gorf). The isoelectric point and molecular weight of the deduced protein were predicted using the ExPASy Molecular Biology Server (web.expasy.org/compute_pi). The nucleotide sequence was translated into the amino acid sequence using the DANMAN 8.08 software package, and the functional domains were predicted by the SMART program (smart.embl-heidelberg.de). Signal peptide and nuclear localization signals (NLS) were predicted using signalP prediction and NLS prediction programs (http://cubic.bioc.columbia.edu/cgi/var/nair). The phylogenetic analysis was conducted using Clustal X2 and MEGA 6 programs. A neighbor-joining (NJ) phylogenetic tree was constructed using MEGA 6 and tested for reliability with 5000 bootstrap replications.

Tissue-specific expression and expression profile of MmQM

The transcripts of *MmQM* in various tissues from healthy *M. meretrix* and its temporal expression in the hepatopancreas after bacterial challenges were detected via RT-qPCR. All of the RT-qPCR assays were performed with SYBR premix ExTaq (Tli RNaseH plus, Takara, Japan) on an ABI 7500 Real-Time Detection System (ThermoFisher, USA). The expression of *MmQM* was normalized to that of the *β-actin* gene for each sample. The primers used in this assay were designed using PerlPrimer 1.1.21, and the information of all primers is shown in Table 1. The relative expression of target gene was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All data are given in terms of relative mRNA expression level as the mean ± standard deviation and were subjected to one-way ANOVA followed by Duncan's multiple range tests by using SPSS 17.0. Differences were considered statistically significant at $P < 0.05$.

Results

cDNA cloning and sequence analysis of MmQM

The full-length *MmQM* cDNA (GenBank Accession No. KU301769) was 819 bp including a 657 bp open reading frame (ORF), a 32 bp 5'-UTR and an 129 bp 3'-UTR with a canonical polyadenylation signal (AATAAA). The nucleotide and deduced amino acid sequences are shown in Fig. 1. The ORF encoded a polypeptide of 218 amino acids with a theoretical molecular mass of

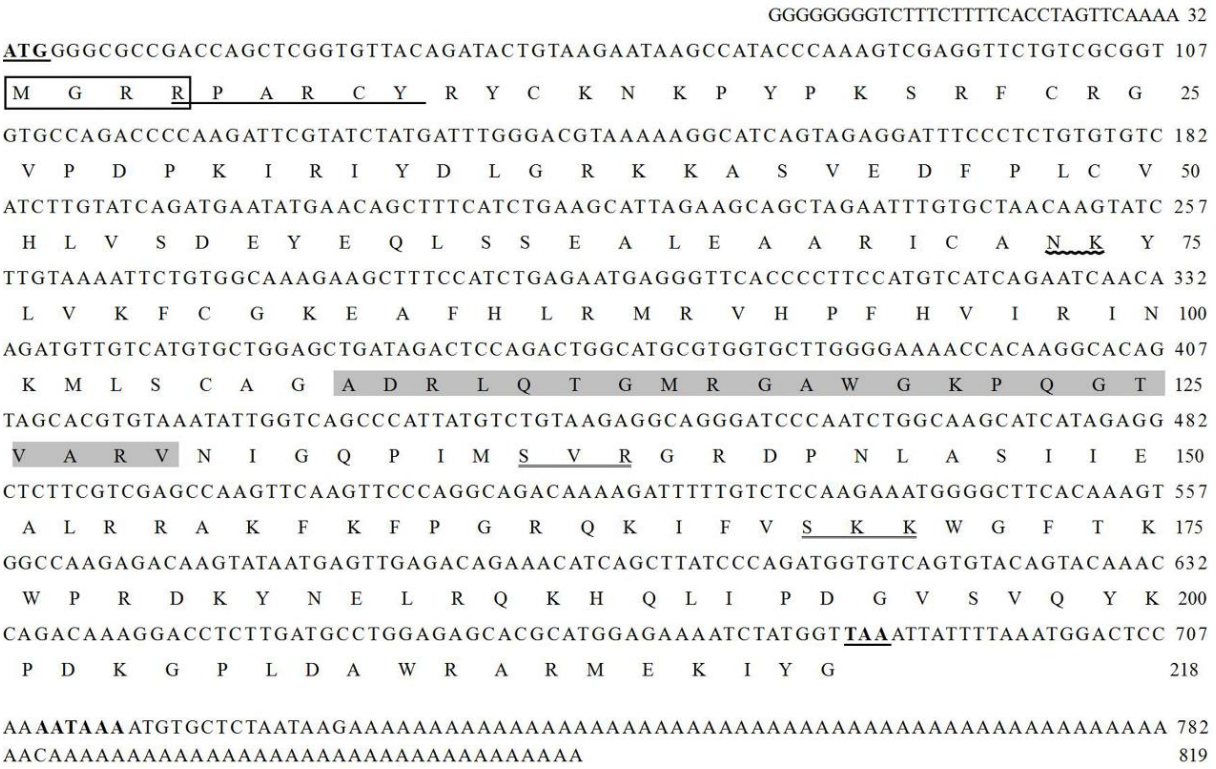

Fig. 1 Nucleotide sequence and deduced amino acid sequence of *MmQM*. The start codon (ATG) and stop codon (TAA) are in bold and underlined. The polyadenylation signal sequence (AATAAA) is in bold. The ribosomal protein L10 signature (ADRLQTGMRGAWGKPQGTVARV) is in a shaded background, and the predicted SH_3-binding motif (RPARCYR) is shaded with a line. The amidation site (MGRR) is boxed, respectively. The protein kinase C phosphorylation sites (SCR and SKK) are double-underlined, and the antibiotic binding site (NK) is in bold and wave-underlined

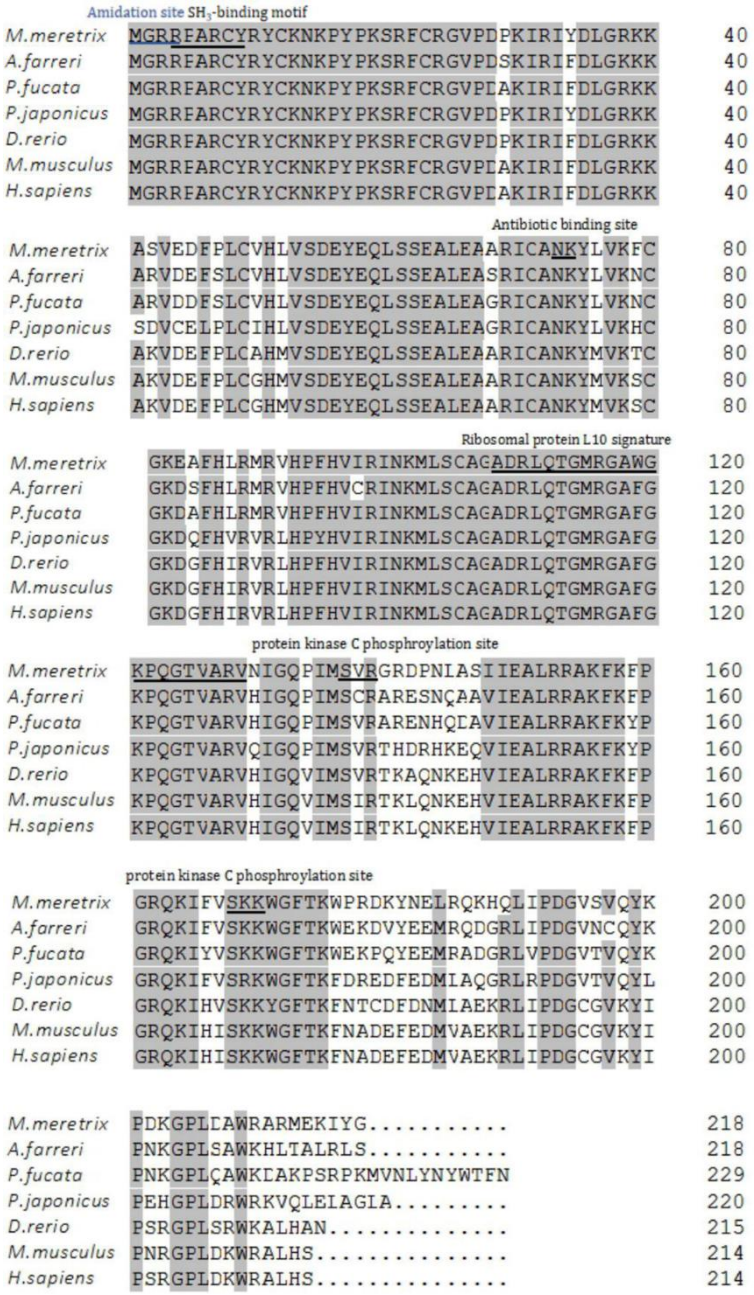

Fig. 2 Multiple alignment of *MmQM* protein with other known vertebrate and invertebrate QM proteins. Analysis was performed using representative QM proteins from *Azumapecten farreri* (AKM12718), *Pinctada fucata* (AAN85578), *Penaeus japonicus* (ABS45569), *Danio rerio* (AAV34163), *Mus musculus* (CAA53061), and *Homo sapiens* (AAB27665) by ClustalW. Identical amino acids in all sequences are highlighted by a shaded background. The characteristic domains of *Mm*QM are bold-underlined and named on the domain

25.3 kDa and an isoelectric point at 10.2. Several characteristic motifs were revealed in the deduced amino acid sequence of *Mm*QM, including an amidation site (^{33}MGRR^{36}), an SH_3-binding motif (^{4}RPARCY^{9}), an antibiotic binding site (^{73}NK^{74}), two protein kinase C phosphorylation sites (^{137}SVR^{139}, ^{168}SKK^{170}) and a ribosomal protein L10 signature (^{108}ADRLQTGMRGAWGKPQGTVARV^{129}). Neither a signal peptide nor a nuclear localization signal (NLS) was detected in *MmQM*.

Homologous and phylogenetic analysis of MmQM

The *MmQM* amino acid identity and similarity percentages were conducted using the BLASTX program, and the deduced amino acid sequence shared high identity to representative QM sequences belonging to the Mollusca. Furthermore, the deduced amino acid sequence shared high identities to representative QM sequences belonging to the Mollusca QM protein sequences from *C. farreri* (84.0%) and *P. fucata* (83.0%) and had an

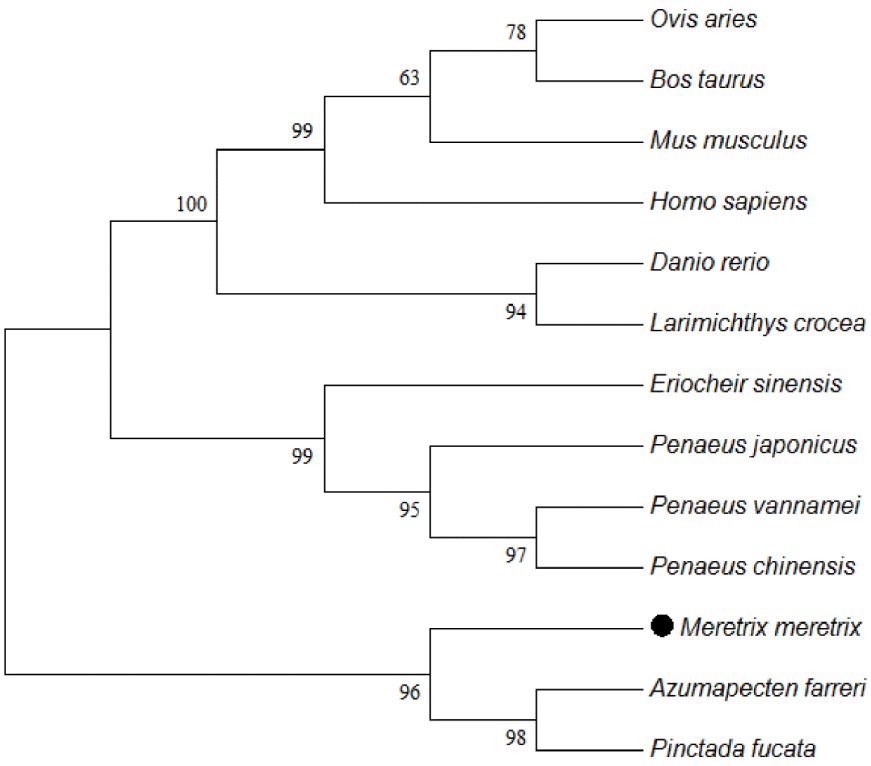

Fig. 3 Phylogenetic relationship of *MmQM* with known QM proteins. The tree is based on an alignment corresponding to full-length amino acid sequences, using ClustalX2 and MEGA6. The number at each node indicates the percentage of boot-strapping after 5000 replications. All of the protein sequences, excluding *Mm*QM, were downloaded from Genbank. *O. aries* (ABV64839), *B. taurus* (NP_777185), *M. musculus* (CAA53061), *H. sapiens* (AAB27665), *D. rerio* (AAV34163), *L. crocea* (ACS93602), *E. sinensis* (ATO74510), *P. japonicus* (ABS45569), *P. vannamei* (AGA16579), *P. chinensis* (ANH58179), *M. meretrix* (ANG56314), *A. farreri* (AKM12718), *P. fucata* (AAN85578)

overall similarity to the sequences from *P. japonicus* (78.0%), *Danio rerio* (78.0%), *Mus musculus* (76.0%), and *Homo sapiens* (77.0%).

To identify the evolutionary conservation of characteristic motifs and active sites of QM proteins, multiple alignment was performed using different QM homologues, including mollusks (*C. farreri*, *P. fucata*), arthropods (*P. japonicus*), fish (*D. rerio*), and mammals (*M. musculus*, *H. sapiens*). The results demonstrated that the N-terminal and middle regions of *MmQM* were more conserved than the C-terminal region (Fig. 2). Moreover, it was revealed that an SH_3-binding motif, two protein kinase C phosphorylation sites, an antibiotic binding site, an amidation site and a ribosomal protein L10 signature were highly conserved during the evolution of QM proteins.

A phylogenetic tree was constructed by the NJ method to investigate the position of *MmQM* in the context of evolution of the QM family (Fig. 3). All of the QM proteins can be clearly recovered in three separate clusters that correspond to mollusks, arthropods and vertebrates in the phylogenetic tree. The vertebrate group included separate clusters belonging to mammalians (*Oivs aries*, *Bos taurus*, *M. musculus*, *H. sapiens*) and fish (*D. rerio*, *L. crocea*). The invertebrate consisted of mollusks (*M. meretrix*, *C. farreri*, *P. fucata*) and arthropods (*Eriocheir sinensis*, *P. japonicus*, *Penaeus vanname*i, *Penaeus chinensis*) that showed two independent clusters. Within the mollusk cluster, *MmQM* formed a single lineage with a high bootstrap value of 96%. These results indicate that *MmQM* is a member of the QM protein subfamily but that it is remarkably different from other known QM proteins.

Tissue-specific expression of MmQM

The relative expression level of *MmQM* mRNA in various tissues including hemocytes, adductor muscle, foot, mantle, gill and hepatopancreas was determined by qRT-PCR with β-actin as an internal control (Fig. 4). The *MmQM* mRNA was found to be constitutively expressed in all of the examined tissues. The hepatopancreas and hemocytes displayed the highest and lowest *MmQM* expression levels, respectively. The fold expression was calculated using the hemocytes as the basis for comparison. The adductor muscle, foot, mantle, gill and hepatopancreas demonstrated 3.59, 4.88, 15.14, 19.38 and 28.38-fold higher *MmQM* expression levels than the hemocytes ($P < 0.05$), respectively. In addition, the expression levels in all tissues were significantly different from each other ($P < 0.05$).

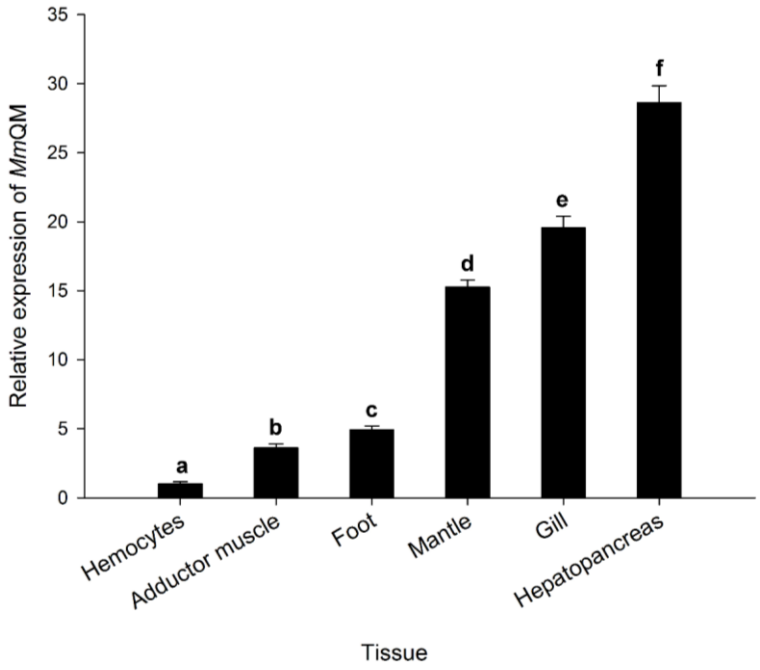

Fig. 4 Tissue expression level of *MmQM*. The fold expression of each tissue was normalized with that of hemocytes to determine the tissue-specific expression of *MmQM*. Data are presented as mean ± SD for five replicates of RT-qPCR reactions using pooled tissue from three individuals. Different letters indicate significant differences ($P < 0.05$) determined by one-way ANOVA and Duncan's multiple comparisons

Temporal expression profile of MmQM *in hepatopancreas after bacterial stimulation*

Transcriptional changes of *MmQM* in the hepatopancreas after immune challenge at different times were normalized to 0 h, and the temporal expression patterns of *MmQM* are shown in Fig. 5. After cultivation with seawater that contained *V. splendidus*, the expression level of *MmQM* exhibited a significant increase at 3 h postchallenge ($P < 0.05$) and reached its highest level, representing a 7.50-fold higher level at 3 h than that in the control group. The expression level of *MmQM* gradually decreased from 6 h to 24 h, demonstrating 3.55 to 1.17-fold higher level than in the control group and had a fluctuation at 48h where it increased to 3.41-fold higher than the control group and then normalized at 96 h. The expression levels of *MmQM* did not exhibit any significant changes in the control group over time.

Discussion

Since the first QM gene was identified from humans, the QM gene has been found in a wide range of living organisms, especially in several kinds of marine invertebrate. All of these identified QM proteins of known marine invertebrates contain conserved signature sequences, such as an SH_3-binding motif, a ribosomal protein L10 signature, an antibiotic binding site, a protein kinase C phosphorylation site, an N-myristoylation site, an N-glycosylation site, an N-acylation site, and an acyl-amidation site (Oh *et al.*, 2010; Liu *et al.*, 2014; Chen *et al.*, 2015). In the present study, the cloned *MmQM* was subsequently found to contain these characteristic features of QMs. Thus, *MmQM* was regarded as a candidate gene that included several characteristic motifs from marine invertebrate QMs and may have a similar function as QMs from other invertebrates. Phylogenetic analysis showed that *MmQM* was grouped together with the QMs from other mollusks, while QMs from fish and mammals were clustered into the same subgroup, and these results were in line with the traditional classification (Fig. 3). These results agree with those deduced from the homologous sequence analysis of *MmQM*.

Different species display tissue differences of QM at the transcript level, such as QM in *C. farreri* and *H. discus* (Oh *et al.*, 2010; Chen *et al.*, 2015). In the present study, *M. meretrix* QM was expressed in all of the examined tissues, including hemocytes, gill, foot, mantle, muscle, and hepatopancreas. The highest RNA expression levels of QM were observed in the hepatopancreas, followed by the gill. Lacking independent immune organs, in shellfish the hepatopancreas is the main immunity and detoxification organ, and the gill is part of the mucosal system, functioning as the first line of defense against pathogens in lower animals (Wang *et al.*, 2019). Therefore, the high mRNA expression levels of *MmQM* in the hepatopancreas and gill indicate that *MmQM* could participate in the innate immune system of *M. meretrix*.

Increasing evidence has shown that QM-like genes have important immune functions in a wide range of organisms (Marty *et al.*, 1993; Lillico *et al.*,

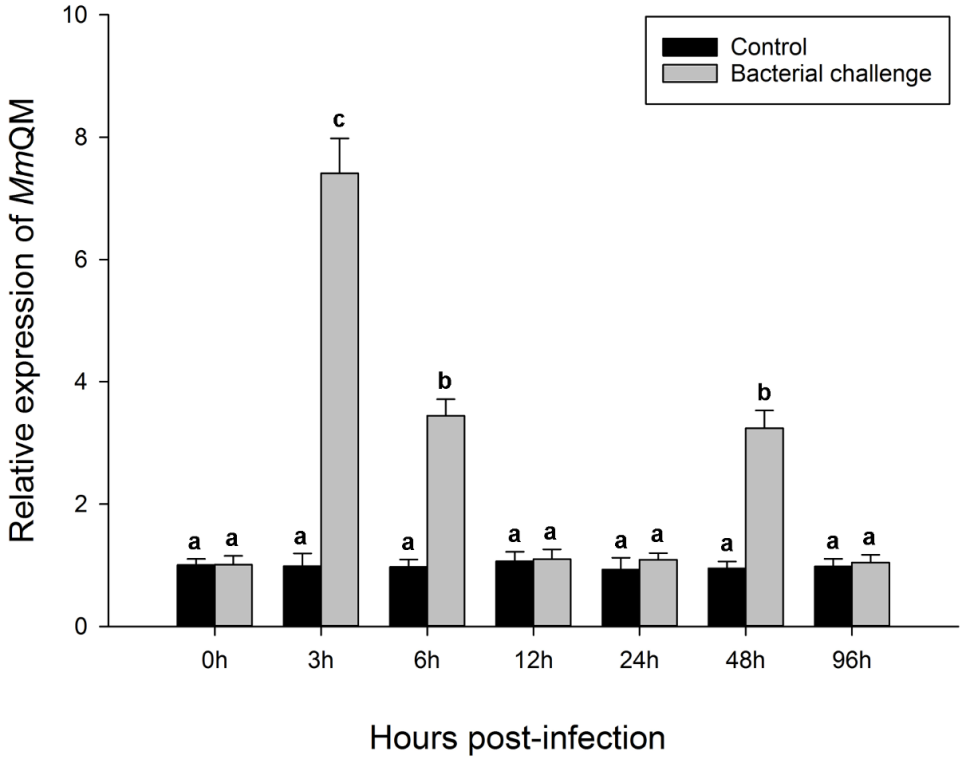

Fig. 5 Temporal expression analysis of *MmQM* in hepatopancreas post bacterial stimulation. Relative expression level of *MmQM* at each time point was normalized with the expression level at the beginning of the challenge test (0h). Data are presented as mean ± SD for five replicates of RT-qPCR reactions using pooled tissue from three individuals at each time point. Different letters indicate significant differences ($P < 0.05$) determined by one-way ANOVA and Duncan's multiple comparisons

2002; Zhang *et al*., 2004; Wen *et al*., 2005; Xu *et al*., 2008; Bhardwaj *et al*., 2010; Oh *et al*., 2010; Liu *et al*., 2014). To further investigate the role of *MmQM* in immune responses, the present research detected the temporal expression profiles of QM in response to a bacterial challenge. The expression of the QM gene in the hepatopancreas was upregulated post bacterial challenge in *M. meretrix*. Similarly, the QM gene in the hemolymph is upregulated after infection with acute viral necrobiotic virus and *V. anguillarum* in *C. farreri* (Chen *et al*., 2015). After infection with *V. anguillarum*, the QM gene of *L. vannamei* is upregulated in various tissues, such as the hemolymph, hepatopancreas, gill, heart, intestine, and muscle (Liu *et al*., 2014). Based on these results, we demonstrated that QM expression can be induced by pathogen attack, further verifying that QM may play a positive role in the response to various pathogens and act as an essential component in host defense system. Specially, the upregulation of the expression level of *MmQM* at 48h, which is different from the other results, suggests that *MmQM* might be involved in multiple immune response processes or be regulated by multiple immune response pathways and confirms the opinion that the invertebrate immune systems are not homogeneous, not simple and are not well understood (Loker *et al*., 2004).

In summary, we identified a tumor suppressor QM-like gene from *M. meretrix* and successfully cloned the cDNA sequence of *MmQM*, whose deduced amino acid sequence was structurally related to the QM protein family. Tissue expression analysis indicated that *MmQM* was expressed in all of the examined tissues and its transcriptional regulation was different among the tissue types. Specifically, the highest and lowest expression levels of *MmQM* were displayed in the hepatopancreas and hemocytes, respectively. In addition, the transcriptional expression of *MmQM* in the hepatopancreas appears be induced by bacterial challenges. Overall, these results indicate that *MmQM* is involved in immune responses and has an essential functional role.

Acknowledgements

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References

Bhardwaj PK, Ahuja PS, Kumar S. Characterization of gene expression of QM from *Caragana jubata*, a plant species that grows under extreme cold. Mol. Biol. Rep. 37: 1003-1010, 2010.

Chen G, Zhang C, Wang Y, Wang Y, Guo C, Wang C. Molecular characterization and immune response expression of the QM gene from the scallop *Chlamys farreri*. Fish Shellfish Immunol. 45: 543-550, 2015.

Chen H, Yang X, Tang T, Li J, Liu B, Liu F, *et al*. The involvement of cysteine-rich intestinal protein in early development and innate immunity of Asiatic hard clam, *Meretrix meretrix*. Fish Shellfish Immunol. 40: 435-440, 2014.

Han F, Xiao S, Zhang Y, Wang Z. Molecular cloning and functional characterization of a QM protein in large yellow croaker (*Larimichthys crocea*). Fish Shellfish Immunol. 44: 187-194, 2015.

Inada H, Mukai J, Matsushima S, Tanaka T. QM is a novel zinc-binding transcription regulatory protein: Its binding to c-Jun Is regulated by zinc ions and phosphorylation by protein kinase C. Biochem. Biophy. Res. Commun. 230: 331-334, 1997.

Koller HT, Klade T, Ellinger A, Breitenbach M. The yeast growth control gene GRC5 is highly Homologous to the mammalian putative tumor suppressor gene QM. Yeast 12: 53-65, 1996.

Li HJ, Liu WD, Gao XG, Zhu D, Wang J, Li YF, *et al*. Identification of host-defense genes and development of microsatellite markers from ESTs of hard clam *Meretrix meretrix*. Mol. Biol. Rep. 38: 769-775, 2011.

Lillico SG, Mottram JC, Murphy NB, Welburn SC. Characterisation of the QM gene of *Trypanosoma brucei*. FEMS Microbiol. Lett. 211: 123-128. 2002.

Liu B, Dong B, Tang B, Zhang T, Xiang J. Effect of stocking density on growth, settlement and survival of clam larvae, *Meretrix meretrix*. Aquaculture 258: 344-349, 2006.

Liu R, Qiu LM, Yu ZA, Zi J, Yue F, Wang LL, Zhang H, Teng WM, Liu XF, Song LS. Identification and characterisation of pathogenic *Vibrio splendidus* from Yesso scallop (*Patinopecten yessoensis*) cultured in a low temperature environment. J. Invertebr. Pathol. 114: 144-150, 2013.

Liu Y, Qian Z, Qu R, Wang X, He S, Hou F, *et al*. Molecular characterization, immune responsive expression and functional analysis of QM, a putative tumor suppressor gene from the Pacific white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 37: 1-10, 2014.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25: 402-408, 2001.

Loker ES, Adema CM, Zhang SM, Kepler TB. Invertebrate immune systems--not homogeneous, not simple, not well understood. Immun. Rev. 198: 10-24, 2004.

Marty I, Brugidou C, Chartier Y, Meyer Y. Growth related gene expression in *Nicotiana tabacum* mesophyll protoplasts. The Plant J. 4: 265-278, 1993.

Mills AA, Mills MJ, Gardiner DM, Bryant SV, Stanbridge EJ. Analysis of the pattern of QM expression during mouse development. Differentiation 64: 161-171, 1999.

Oh C, De Zoysa M, Nikapitiya C, Whang I, Kim YC, Kang DH, *et al*. Tumor suppressor QM-like gene from disk abalone (*Haliotis discus discus*): molecular characterization and transcriptional analysis upon immune challenge. Fish Shellfish Immunol. 29: 494-500, 2010.

Tang B, Liu B, Wang G, Zhang T, Xiang J. Effects of various algal diets and starvation on larval growth and survival of *Meretrix meretrix*. Aquaculture, 254: 526-533, 2006.

Wang C, Huan P, Yue X, Yan MC, Liu BZ. Molecular characterization of a glutathione peroxidase gene and its expression in the selected Vibrio-resistant population of the clam Meretrix meretrix, Fish Shellfish Immunol, 30:1294-1302, 2011.

Wang MQ, Wang BJ, Jiang KY, Liu M, Shi XW, Wang L. A mitochondrial manganese superoxide dismutase involved in innate immunity is essential for the survival of Chlamys farreri. Fish Shellfish Immunol. 72: 282-290, 2018.

Wang Y, Wang MQ, Wang BJ, Liu M, Jiang KY, Hou XG, *et al*. A preliminary attempt to explore the potential functions of a tetraspanin gene (MmTSPAN) in the innate immunity of hard clam *Meretrix meretrix*: Sequence features and expression profles. Fish Shellfish Immunol. 88: 135-141, 2019.

Wen Y, Shao JZ, Pan XX, Xiang LX. Molecular cloning, characterization and expression analysis of QM gene from grass carp (*Ctenopharyngodon idellus*) homologous to Wilms' tumor suppressor. Comp. Biochem. Physiol. B.141: 356-365. 2005.

Xu JY, Wu SJ, Zhang XB. Novel function of QM protein of shrimp (*Penaeus japonicus*) in regulation of phenol oxidase activity by interaction with hemocyanin. Cell Physiol. Biochem. 21: 473-480, 2008.

Yang Q, Yang Z, Li H. Molecular characterization and expression analysis of an inhibitor of NF-kappaB (IkappaB) from Asiatic hard clam *Meretrix meretrix*. Fish Shellfish Immunol. 31: 485-490, 2011.

Yue X, Liu B, Sun L, Tang B. Cloning and characterization of a hsp70 gene from Asiatic hard clam *Meretrix meretrix* which is involved in the immune response against bacterial infection. Fish Shellfish Immunol. 30: 791-799, 2011.

Yue X, Liu B, Xiang J, Jia J. Identification and characterization of the pathogenic effect of a *Vibrio parahaemolyticus*-related bacterium isolated from clam *Meretrix meretrix* with mass mortality. J. Invertebr. Pathol. 103: 109-115, 2010.

Zhang Y, Huang J, Meng Q, Jiang T, Xie L, Wang Z, *et al*. Molecular cloning and expression of a pearl oyster (*Pinctada fucata*) homologue of mammalian putative tumor suppressor QM. Mar. Biotechnol. 6: 8-16, 2004.

Zhang Y, Zhou Q, Ke CH, Huang HQ. Cloning and expression analysis of γ-aminobutyrate type A receptor-associated protein (GABARAP) in

Asiatic hard clam, *Meretrix meretrix*. Aquaculture 432: 327-335, 2014.

Zhou F, Jiang S, Huang J, Qiu L, Zhang D, Su T. Molecular analysis of the QM gene from *Penaeus monodon* and its expression on the different ovarian stages of development. Mol. Biol. Rep. 38: 1921-1927, 2011.

Zhou XS, Chen C, Li TH, Tang JJ, Zhu BJ, Wei GQ, *et al*. A QM protein from Bombyx mori negatively regulates prophenoloxidase activation and melanization by interacting with Jun protein. Insect Molecular Biology: 1-13, 2019.

Zou L, Liu B. Identification of a serum amyloid A gene and the association of SNPs with *Vibrio*-resistance and growth traits in the clam *Meretrix meretrix*. Fish Shellfish Immunol. 43: 301-309, 2015.

Zou L, Liu B. The polymorphisms of a MIF gene and their association with *Vibrio* resistance in the clam *Meretrix meretrix*. Dev. Comp. Immunol. 62: 116-126, 2016.