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High mobility group box 2 of black rockfish *Sebastes schlegelii*: Gene cloning, immunoregulatory properties and antibacterial effect

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ABSTRACT

High-mobility group box 2 (HMGB2) is a non-histone chromosomal protein that involved diverse functions such as transcriptional regulation and innate immune responses in mammalian. In teleost, very limited studies on HMGB2 proteins have been documented. Black rockfish (*Sebastes schlegelii*) is an economic fish species and cultured worldwide. However, the study of black rockfish about immunology is very scarce. In the present study, a HMGB2 homologue gene (*SsHMGB2*) was identified and characterized in black rockfish. The open reading frame of *SsHMGB2* is 648 bp, and the deduced amino acid sequence of *SsHMGB2* shares 74.4%–91.2% overall sequence identities with the HMGB2 proteins of several fish species. In silico analysis identified several conserved features, including two basic HMG boxes and an acidic C-terminal tail composed of 24 Asp/Glu residues. Expression of *SsHMGB2* occurred in multiple tissues and was upregulated during pathogens infection. Recombinant SsHMGB2 (rSsHMGB2) exhibited apparent binding activities against DNA. *In vivo* studies showed that the expressions of multiple immune-related genes in head kidney were significantly enhanced when black rockfish were treated with rSsHMGB2. Furthermore, rSsHMGB2 reduced pathogen dissemination and replication in fish kidney and spleen. Taken together, these results suggest that SsHMGB2 possesses apparent immunoregulatory properties and played a role in fighting bacterial infection.

1. Introduction

High mobility group box (HMGB) proteins are non-histone chromosomal proteins that play importante roles in DNA transcription, replication, recombination, repair and gene expression regulation along with core histones [1–5]. These proteins are highly conserved and ubiquitously distributed in vertebrates and invertebrates [6,7]. In mammals, HMGB proteins contain four members at present: HMGB1, HMGB2, HMGB3 and a novel member, HMGB4, of which HMGB1-3 share high sequence identities (more than 80%) and are similar in biochemical properties and structural characteristics. For example, HMGB1-3 proteins include three distinct domains, i.e., two tandem HMG boxes in the N-terminal and middle regions (termed box A and box B), and an acidic domain in the C-terminal region [8–12]. In contrast to HMGB1-3, HMGB4 possesses HMG box A and box B but lacks the acidic tail [11].

HMGB1 and HMGB2 are the most extensively studied in humans and mice amongst the four types of HMGB proteins. Both HMGB1 and HMGB2 can non-specifically bind to noncanonical and distorted DNA structures such as single-stranded DNA, double-stranded DNA, supercoiled DNA, and damaged DNA [13,14]. Intracellular HMGB1/HMGB2 could be released extracellularly after stimulation with pathogen-derived molecules and released be able to regulate various immune and inflammatory responses [15–18]. As nuclear proteins, HMGB1/HMGB2 are known to regulate various cellular activities, including transcription, DNA replication, and repair. They also have the ability of binding to transcription factors such as Hox proteins steroid hormone receptors, p53, and p73, and the binding enhances the transcription and

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recombination activities of their partner proteins [19–21]. It was reported that HMGB2 is secreted by myeloid cells and has mitogenic and chemoattractant activities similar to HMGB1 [22]. Despite the high degree of amino acid sequence similarity between HMGB1 and HMGB2, the two HMGB proteins are difference in some aspects. Contrary to the ubiquitous expression of HMGB1, HMGB2 is restricted mainly to lymphoid organs and testes [23]. HMGB2 seems to have a role in germ cell differentiation [24].

In teleost, studies on HMGB1 proteins are documented in zebrafish, grass carp, red drum, humphead snapper, goldfish, and black rockfish [25]. However, studies on HMGB2 proteins in teleost have only been reported for zebrafish, in which it was found that the HMGB2 gene is highly expressed during embryonic development, and for grass carp and tongue sole, in which it was found that HMGB2 proteins play crucial roles in innate immune defense [26,27]. Black rockfish (*Sebastes schlegelii*) is cultured widely in China, Korea and Japan as an economic fish species. However, the fish has long been suffered from serious diseases at present, and the immune mechanism of black rockfish responses to pathogens infection is very limited. In this study, we identified and characterized a new HMGB2 homologue from black rockfish *Sebastes schlegelii* (SsHMGB2), and examined its biological properties. We found the expression of *SsHMGB2* were induced by pathogens and possessed the property of binding with DNA. SsHMGB2 also exhibited an immunostimulatory effect in fish, which led to enhanced resistance against bacterial infection. Our results indicated that SsHMGB2 play a role in host immune defense against pathogens.

2. Materials and methods

2.1. Ethics statement

The experiments involving live animals were conducted in accordance with the “Regulations for the Administration of Affairs Concerning Experimental Animals” promulgated by the State Science and Technology Commission of Shandong Province. The study was approved by the ethics committee of Qingdao Agricultural University.

2.2. Fish

Black rockfish (*Sebastes schlegelii*) (average 8.6 ± 0.3 g) were obtained from a commercial fish farm (Qingdao, Shandong Province, China) and maintained at 15 °C in culture tanks for a week. Before the experiment, fish were randomly sampled and verified to be clinically healthy as reported previously [28,29]. Fish were euthanized with tricaine methanesulfonate (Sigma, St. Louis, USA) as reported previously before tissue collection [30].

2.3. Cloning of SsHMGB2

A cDNA library of black rockfish head kidney and spleen was constructed as described previously [25]. One of the clones was found to contain the partial cDNA of *SsHMGB2* with 5′-untranslated region (UTR) by DNA sequence analysis. The amplifications of 3′-UTR reaction was performed using a SMART™ RACE cDNA amplification kit (Clontech, Indianapolis, IN, USA) as described previously [31], the primers used were listed in Table 1.

2.4. Sequence analysis of SsHMGB2

Homology comparisons were performed with BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast). The information of the deduced amino acid sequence was analyzed with the DNAman software package (Lynnon Biosoft, Quebec, Canada). The cleavage site for the signal peptide and protein domains were analyzed using the SMART program (http://smart.embl-heidelberg.de/). The multiple alignment and phylogenetic tree were constructed with the ClustalW Multiple Alignment program (http://www.ebi.ac.uk/clustalw/) and MEGA 5.0 software package considering 1000 bootstrap hits.

Table 1
PCR primers used in the study.

Primers name	Sequence (5′–3′)
3′Race primers	
3′Race SsHMGB2-F	GTGATGCTGGTAAGAAGGGTGGC
3′Race R1	CTAATACGACTCACTATAGGGC
qRT-PCR primers	
EF1A-F	AACCTGACCACTGAGGTGAAGTCTG
EF1A-R	TCCTTGACGGACACGTTCTTGATGTT
SsHMGB2-F	TGACAACATACATCCCTCCTAAAG
SsHMGB2-R	GCAGAACACGAAGAACGCAGA
TNF13B—F	GGAAAACCTTCAGGAAAGAATACA
TNF13B-R	TGAGGCTCGTCTCCCACC
IL-1β-F	GCATCCGAGGCACAAATCC
IL-1β-R	ACACCCGCTCCACTCAACAG
IL-8-F	CCTCATTTTAATACCACAGG
IL-8-R	ACAAACAAGCACAGACTTCT
Hp-F	GGCAGGGAAAGAGGGAATAG
Hp-R	GGAAGTGTGGATGGAGAAAAA
SAA-F	CTTCCCCGGTGAAGCCTTTA
SAA-R	CCATGCTCATTTGCTCTCTGAT
HSP70—F	CTGTTTGAAGCAATTGAGGGC
HSP70-R	CAGGAGTTTCTGGATTTTAGGGA
ISG15-F	CTACGGCCTGCAGCAAGGAGC
ISG15-R	CCCTGGTCTTGAAGTTGGCCA
Recombinant primers	
rSsHMGB2-F	ACAAAGGATCCAAATAAGCCAAGAG
rSsHMGB2-R	TTATTCATCA TCGTCATCAT CATCGT

2.5. mRNA expression analysis of SsHMGB2

Quantitative real-time PCR (qRT-PCR) was performed to measure mRNA transcription of *SsHMGB2* under normal conditions in liver, kidney, brain, spleen, heart, muscle, blood, intestine and gills. The black rockfish elongation factor 1α (*SsEF1A*) gene (GenBank accession no: KF430623) was the internal reference for normalizing the qRT-PCR results [32–35]. Assays were performed using a LightCycler 96 system (Roche Applied Science, North Carolina, USA) with the SYBR ExScript qRT-PCR Kit (Takara, Dalian, China) as described previously [36]. The primers for qRT-PCR were listed in Table 1. The results were analyzed according to the comparative threshold cycle method ($2^{-\Delta\Delta Ct}$). The experiment was performed for three times, each time with five fish.

Bacterial infection was performed as reported previously [37]. *Vibrio anguillarum* and *Edwardsiella tarda* TX1 were cultured in Luria-Bertani (LB) broth to mid-logarithmic phase. The cells were washed and resuspended in phosphate-buffered saline (PBS, 0.14 M sodium chloride, 3 mM potassium chloride, 8 mM disodium hydrogen phosphate dodecahydrate, 1.5 mM potassium phosphate monobasic, pH 7.4) to 10^7 colony forming units (CFU)/ml. Three groups (30 individuals/group) of black rockfish were injected intraperitoneally (i.p.) with 100 µl V. *anguillarum*, *E. tarda* or PBS. At 0 h, 4 h, 8 h, 12 h, 24 h and 48 h post-injection, five individuals from each group were randomly sampled. *SsHMGB2* expressions in liver, spleen and head kidney of the fish were determined by qRT-PCR as above.

2.6. Expression and purification of recombinant protein of SsHMGB2

The mature peptide without signal peptide of SsHMGB2 was amplified with specific primers (Table 1). The PCR products were ligated with the expression vector peasy-Blunt E1 with his-tag (Transgen, Beijing, China). After the recombinant plasmid (pBSsHMGB2) was transformed into Transetta (DE3) (Transgen, Beijing, China), recombinant protein (rSsHMGB2) was expressed, purified and concentrated according to a previous description [25]. The purified protein, his-tagged rSsHMGB2, was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of the protein was measured with the Bradford method using bovine serum albumin (BSA) as the standard.

2.7. Effect of rSsHMGB2 on DNA fragments

The coding sequence of *SsHMGB2* was amplified to detect the DNA-binding ability of rSsHMGB2 according to methods reported previously [25]. The concentration of *SsHMGB2* fragments was measured at 260 nm using a Nanodrop 2000 spectrophotometer (Nano-drop Technologies, Wilmington, DE). *SsHMGB2* fragments (100 ng) were mixed with different concentrations of rSsHMGB2, heat-denatured rSsHMGB2 or PBS in a total volume of 20 μl. The mixture was incubated at room temperature for 1 h and subjected to agarose gel electrophoresis.

2.8. In vivo effect of rSsHMGB2

Three groups (15 individuals/group) of black rockfish were injected intraperitoneally (i.p.) with 15 μg/fish of rSsHMGB2 or recombinant suppressor of cytokine signaling 3 (rSmSOCS3), which was reported previously, purified and reconstituted under the same conditions as rSsHMGB2, then used as a negative control [38]. To determine the expression of immune genes including interleukin (IL) -1β, IL-8, tissue necrosis factor (TNF) 13B, haptoglobin (Hp), serum amyloid A (SAA), heat shock protein 70 (HSP70) and ISG15 (an interferon-stimulated gene), total RNA was extracted from the head kidney tissue after 12 h of rSsHMGB2 treatment and qRT-PCR was used to analyze the expression of immune genes as described above. The experiment was performed three times. The PCR primers of the immune genes are listed in Table 1.

To determine the antibacterial effect, at 2 h post rSsHMGB2 treatment, the fish were infected with 100 μl V. *anguillarum* (5×10^7 CFU/ml) by intraperitoneal injection. The kidney and spleen were taken under aseptic conditions at 12 h and 24 h post-infection. After the tissues were homogenized in PBS, the homogenates were diluted serially in PBS and plated in triplicate on LB agar plates. The plates were incubated, and the colonies on the plates were counted. The experiments were performed three times.

2.9. Statistical analysis

All statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Data were analyzed using analysis of variance (ANOVA), and statistical significance was defined as $P < 0.05$.

3. Results

3.1. Characterization of the sequence of SsHMGB2

Sequence analysis showed that the full-length cDNA of SsHMGB2 contains a 5' -untranslated region (UTR) of 48 bp, an open reading frame (ORF) of 648 bp, and a 3' -UTR of 532 bp (Fig. 1). The cDNA terminates with a poly-A tail, which is preceded by a polyadenylation signal, AATAAA, at 12 bp upstream of the poly-A sequence. The deduced amino acid sequence of SsHMGB2 is 215 residues in length and terminated with a stretch of 24 D/E residues. The protein is predicted by a theoretical isoelectric point of 7.93 and a molecular mass of 24.2 kDa. In silico analysis showed that SsHMGB2 possesses no apparent signal peptide sequence and contains two HMG boxes formed by residues 7 to 79 and 95 to 165, and an acidic C-terminal tail (Fig. 1). The two HMG boxes are rich in positively charged amino acids and have pI values of 10.38 and 10.19, respectively. BLAST analysis showed that SsHMGB2 shares 74.4%–91.2% overall sequence identities with the HMGB2 of a number of teleost species including *Anoplopoma fimbria*, *Lates calcarifer*, *Oreochromis niloticus*, *Danio rerio*, *Oncorhynchus mykiss* (Fig. 2). The overall sequence identity between SsHMGB2 and human HMGB2 is 66.0%. The HMG box domains possess relatively stronger conservative property than C-terminal acidic tail, and the length of which varies according to different species. A neighbor-joining phylogenetic tree was constructed based on the amino acid sequences of HMGB2, and the result showed SsHMGB2 is closest to the HMGB2 of *A. fimbria* (Fig. 3).

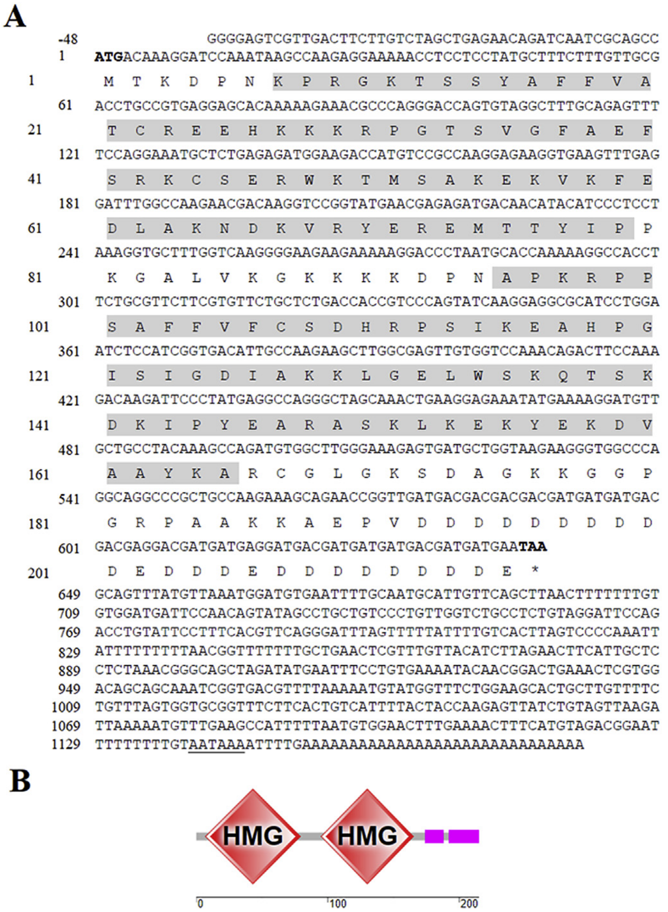

Fig. 1. Sequence analysis of *SsHMGB2*. (A) The nucleotide and deduced amino acid sequences of *SsHMGB2*. The nucleotides and amino acids are numbered along the left margin. In the DNA sequence, the translation start and stop codons are in bold, and the putative polyadenylation signal (aataaa) is underlined. In the amino acid sequence, two conserved HMG domain are shaded in gray. (B) The schematic of protein motifs of *SsHMGB2*. The HMG domains are indicated with orange red diamond, two low complexities are showed with pink rectangle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Expression of SsHMGB2 under normal physiological conditions

Under normal physiological conditions, qRT-PCR analysis showed that *SsHMGB2* expression distributed in all the examined tissues, and with an increasing order, in muscle, brain, spleen, liver, blood, heart, head kidney, gill, and intestine (Fig. 4).

3.3. Expression of SsHMGB2 in tissues after pathogenic challenge

To examine the effect of bacterial infection on *SsHMGB2* expression, black rockfish were challenged with the fish pathogens *V. anguillarum* and *E. tarda*. *SsHMGB2* expression in liver, spleen, and kidney were analyzed by qRT-PCR at 0, 4, 8, 12, 24, and 48 hpi (hours post-infection, hpi). The results showed that *SsHMGB2* expression in liver induced by *V. anguillarum* infection significantly increased at 8, 12, and

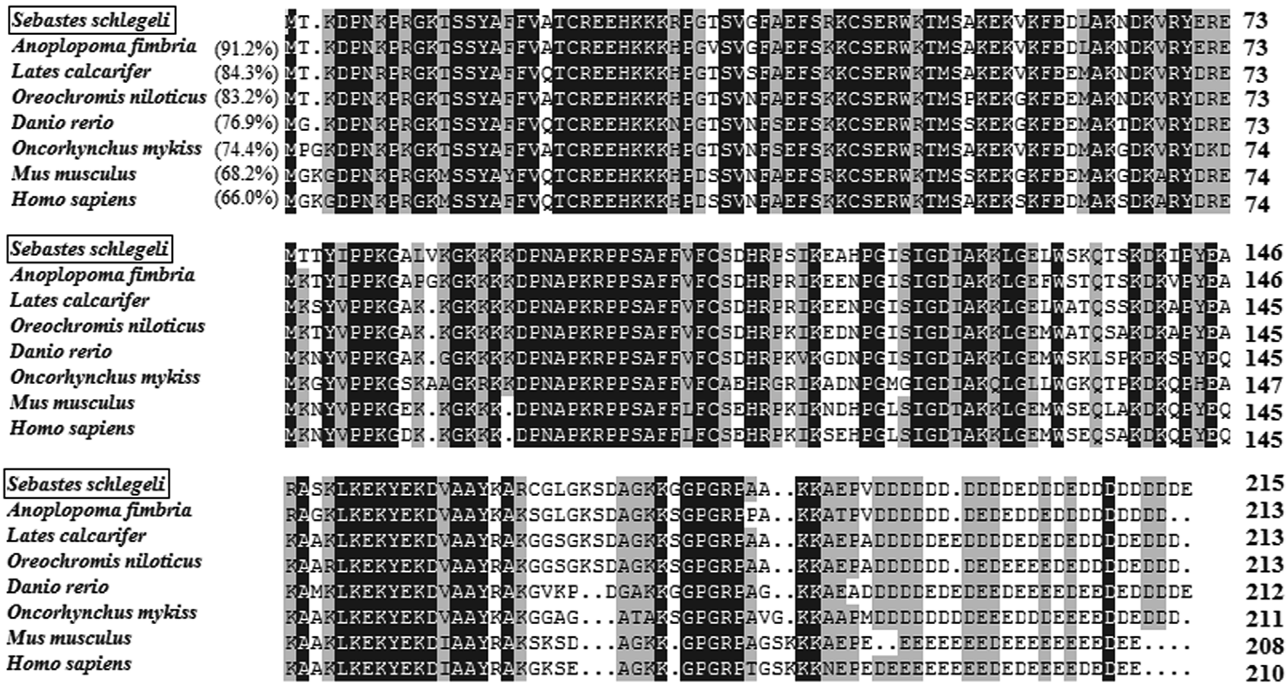

Fig. 2. Alignment of the deduced amino acid sequences of *SsHMGB2* homologues. The percentage number in the bracket following each species name represents the overall sequence identity between *SsHMGB2* and the HMGB2 of the specified species. Dots denote gaps introduced for maximum matching. The consensus residues are in black, the residues that are ≥75% identical among the aligned sequences are in gray. The Gen Bank accession numbers of the aligned sequences are as follows: *Anoplopoma fimbria*, ACQ58903.1; *Lates calcarifer*, XP_018556485.1; *Oreochromis niloticus*, XP_005461309.1; *Danio rerio*, NP_001032501.1; *Oncorhynchus mykiss*, XP_021429142.1; *Mus musculus*, NP_032278.1; *Homo sapiens*, NP_001124161.1.

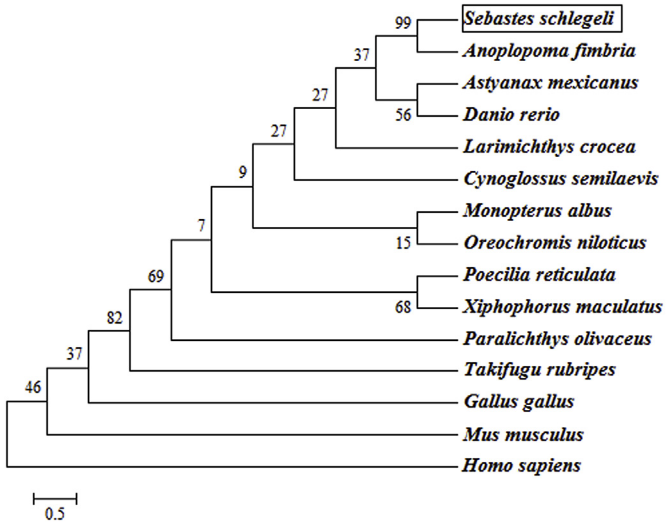

Fig. 3. Phylogenetic analysis of *SsHMGB2* homologues. The phylogenetic tree was constructed with the neighbor-joining algorithm using the Mega 4.0 program based on the multiple sequence alignment. The numbers at the forks indicate the bootstrap values.

24 hpi with peaked expression at 12 hpi (17.9-fold), and fell back to the normal level at 48 hpi. *SsHMGB2* expression in spleen and kidney induced by *V. anguillarum* infection significantly increased at 4, 8, 12, and 24 hpi, with peaked expression at 8 and 12 hpi (23.2- and 21.4-fold), respectively (Fig. 5A). Upon infection of *E. tarda*, the expression of *SsHMGB2* in liver was significantly upregulated at all the examined times, peaked at 8 hpi (29.04-fold). The expression level of *SsHMGB2* in spleen was remarkably enhanced at 4, 8, 12, and 24 hpi by *E. tarda* infection, with the highest level occurring at 12 hpi (34.6-fold). In head kidney, except 4 and 48 hpi, *SsHMGB2* expression was significantly induced by *E. tarda* challenge at other three times, with the maximum induction occurring at 12 hpi (13.9-fold) (Fig. 5B).

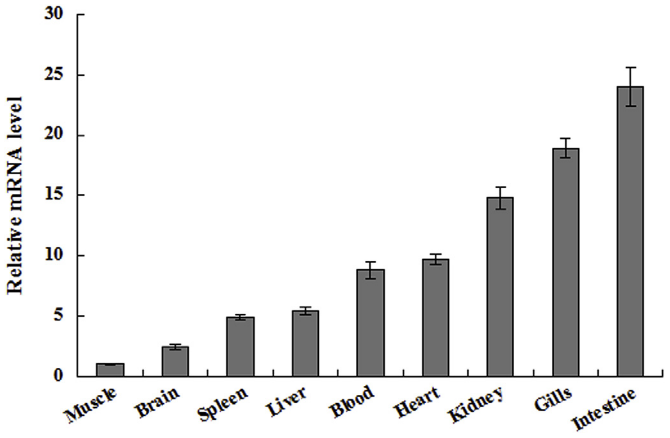

Fig. 4. *SsHMGB2* expression in fish tissues under normal physiological conditions. *SsHMGB2* expression in the muscle, brain, spleen, liver, blood, heart, kidney, gill, and intestine of rock blackfish were determined by quantitative real time RT-PCR. The expression level of *SsHMGB2* in muscle was set as 1. Values are shown as means ± SEM (N = 3). N, the number of times the experiment was performed.

3.4. Effect of rSsHMGB2 on DNA binding

To investigate the effect of rSsHMGB2 on DNA binding, rSsHMGB2 was purified from *E. coli* as a native His-tagged protein. SDS-PAGE analysis revealed a single band with an apparent molecular mass matching that predicted for rSsHMGB2 (Fig. S1). To detect the DNA-binding ability, purified rSsHMGB2, heat-denatured rSsHMGB2 or PBS was incubated with *SsHMGB2* DNA fragments. After incubation, the protein-DNA complexes were subjected to electrophoresis. The results

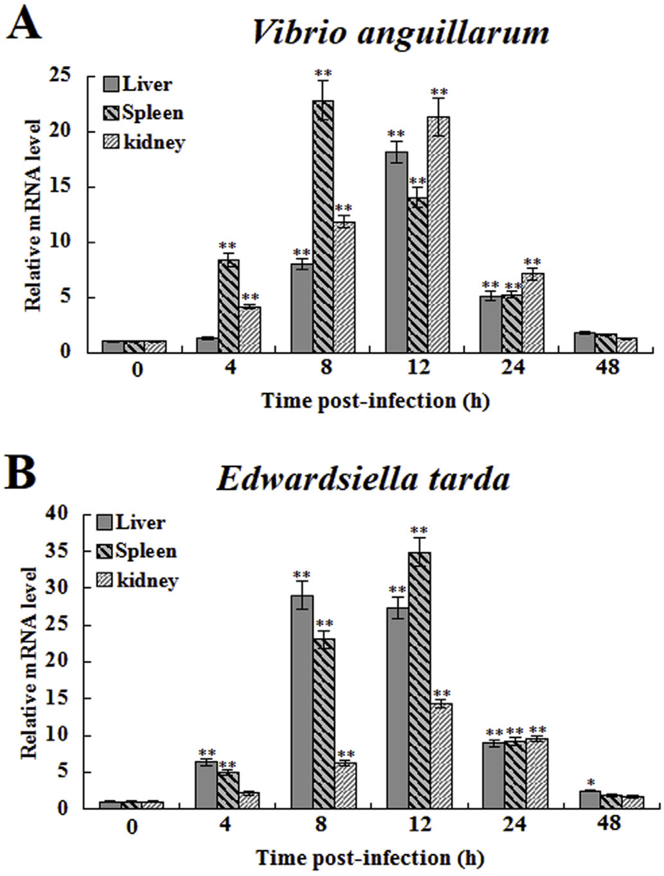

Fig. 5. *SsHMGB2* expression in response to bacterial challenge. Black rockfish were infected with *Vibrio anguillarum* and *Edwardsiella tarda*, and *SsHMGB2* expression in liver, spleen, and head kidney were determined by quantitative real time RT-PCR at different times of infection. In each case, the expression level of the control fish was set as 1. Values are shown as means ± SEM ($N = 3$). N, the number of times the experiment was performed. $^{**}P < 0.01$,$^{*}P < 0.05$.

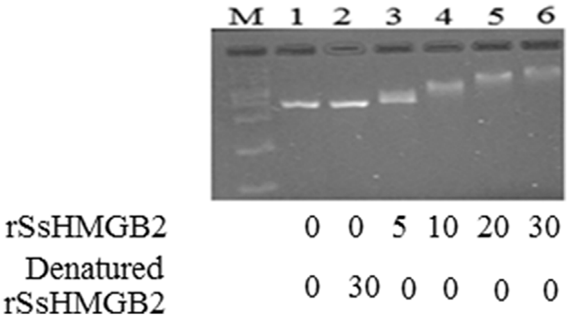

Fig. 6. Binding of rSsHMGB2 to DNA fragments. *SsHMGB2* DNA fragments were mixed with purified rSsHMGB2 (lanes 3, 4, 5 and 6), denatured rSsHMGB2 (lane 2) or PBS (lane 1) and incubated for 1 h at room temperature. After the reaction, the samples were run on a DNA agarose gel.

showed that the rSsHMGB2 could retard the migration of *SsHMGB2* DNA in a dose-dependent manner. In contrast, incubation with heat-denatured rSsHMGB2 or PBS caused no retardation of DNA migration (Fig. 6).

3.5. Effect of rSsHMGB2 on expression of immune-related genes

To determine the pro-inflammatory effect of rSsHMGB2, total RNA was extracted from the head kidney tissue after 12 h of rSsHMGB2 treatment and qRT-PCR was used to analyze the expression of immune genes. qRT-PCR analysis showed that compared to treatment with PBS, treatment with rSsHMGB2 significantly induced expression of the immune genes TNF13B, IL-1β, IL-8, Hp, SAA, Hsp70, and ISG15 (Fig. 7).

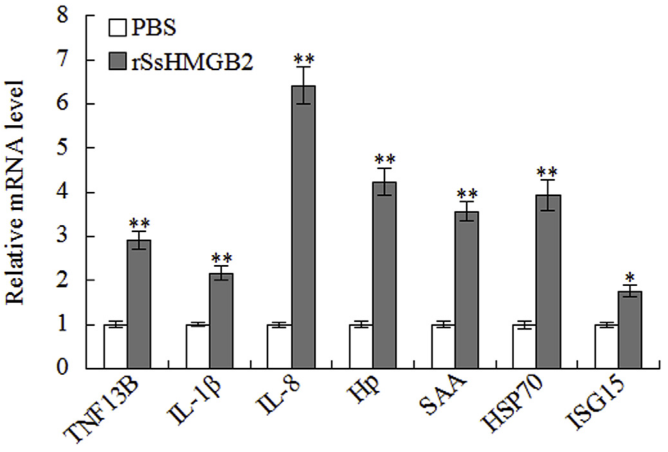

Fig. 7. Immune gene expression induced by rSsHMGB2. Black rockfish were treated with rSsHMGB2 or PBS for 2 h, and the mRNA expression of immune relevant genes was determined by quantitative real time qRT-PCR. For convenience of comparison, the expression level of PBS treatment were set as 1. Values are shown as means ± SEM ($N = 3$). N, the number of times the experiment was performed. $^{**}P < 0.01$,$^{*}P < 0.05$.

3.6. Effect of rSsHMGB2 on bacterial infection

To examine whether rSsHMGB2 had any effect with respect to immune defense against pathogen invasion, black rockfish were pre-administered with rSsHMGB2, rSmSOCS, which was used as a control protein for rSsHMGB2, or PBS, before being infected with *V. anguillarum*, and bacterial loads in kidney and spleen were determined at 12 h and 24 h post-infection. The results showed that the bacterial numbers were significantly reduced in rSsHMGB2-treated fish at 12 h and 24 h in kidney and spleen, whereas the bacterial numbers in rSmSOCS-treated fish were comparable to those in the control fish treated with PBS (Fig. 8). Similar results were observed when fish administered with rSsHMGB2 or rSmSOCS were infected by *E. tarda* (data not shown).

4. Discussion

In our previous work, we had identified high-mobility group box 1 gene from black rockfish (*SsHMGB1*) and analyzed the biological function [25]. In this study, we cloned an HMGB2 homologue from black rockfish, *SsHMGB2*, and examined its expression and biological property. *SsHMGB2* shares over 74.4%–91.2% sequence identify with teleost HMGB2 and 66.0% sequence identify with human HMGB2, and contains two HMG boxes and a C-terminal acidic tail formed by 24 Asp/Glu residues. A phylogenetic analysis indicated that SsHMGB2 was clustered with the HMGB2 from *A. fimbria*. The high sequence identity, together with the phylogenetic analysis and the structural features, indicated that SsHMGB2 is a new member of the vertebrate HMGB2 subfamily.

In mice, HMGB2 are expressed at high levels in thymus, lymphoid tissues, and testis [24]. In teleost, HMGB2 expression has been studied in several fish species. In zebrafish, it was found that the HMGB2 gene is abundantly expressed during embryonic development [39], and in tongue sole and grass carp, which showed that HMGB2 expression occurred constitutively in a wide range of tissues, in particular those of immune relevance [26,27]. In this study, we found that *SsHMGB2* expression was ubiquitously distributed in all examined tissues, with a

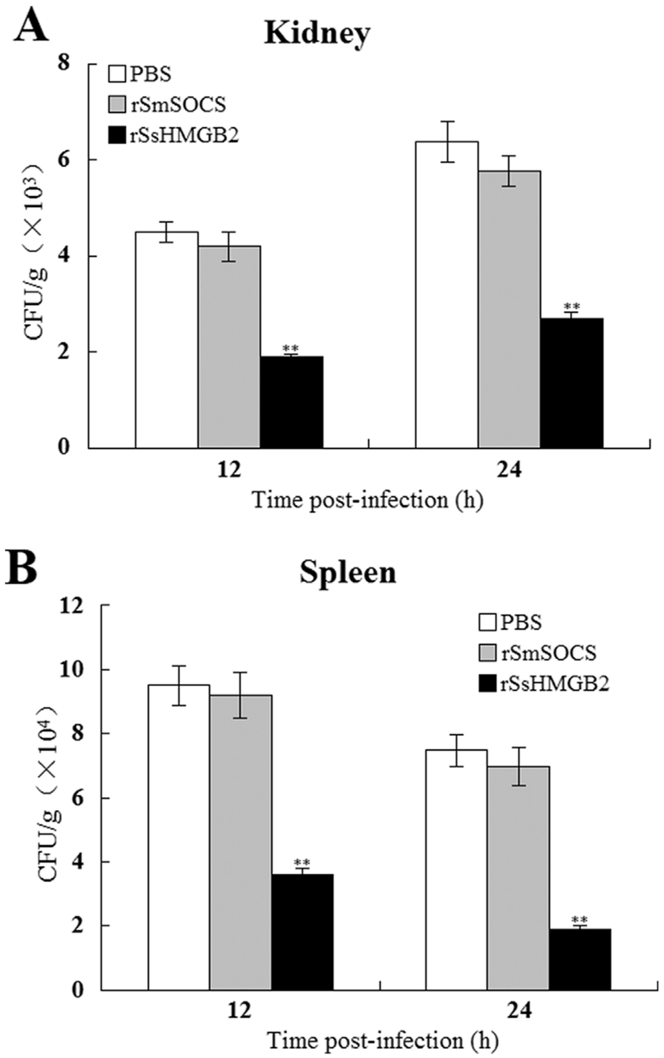

Fig. 8. Effect of rSsHMGB2 on bacterial infection *in vivo*. Black rockfish were administered with rSsHMGB2, rSmSOCS, or PBS before infection with *Vibrio anguillarum*, bacterial loads in kidney (A) and spleen (B) determined by plate count. Values are shown as means ± SEM (N = 3). N, the number of times the experiment was performed. $^{**}P < 0.01$.

higher expression in kidney, gill, and intestine. Furthermore, the expression of *SsHMGB2* in head kidney, spleen, and liver was significantly induced by fish pathogen *V. anguillarum* and *E. tarda*, which is similar to the results of HMGB2 in tongue sole and grass carp [26,27]. These results suggest that SsHMGB2 is probably involved in host immune response against microbial pathogens.

It is known human HMGB1 contains three cysteines, Cys23, Cys45, and Cys106. And Cys23 and Cys45 are located in HMG box A and form a Cys23–Cys45 disulfide bond in the setting of oxidative stress [40]. The proinflammatory activity domain of HMGB1 is localized in the first 20 amino acids of the HMG-box B, which has the most significant cytokine functionality, and HMGB1 Cys106 is located in HMG-box B and is required for TLR4 binding and cytokine release from macrophages [41]. Sequence analysis shows that SsHMGB2 are rich in positively charged amino acid and contains three cysteines (Cys22, Cys44, and Cys107) in two HMG boxes, which suggested that SsHMGB2 maybe have similar cytokine function and binding activity, although the number of amino acid residues is different from mammalian HMGB. In our study, we found SsHMGB2 bore binding activity to DNA, which is the first report in teleost. qRT-PCR analysis showed that HMGB2 protein significantly induced TNF13B, IL-1β, IL-8, Hp, SAA, Hsp70, and ISG15. It is known that TNF13B induces B-cell survival, proliferation, immunoglobulin secretion and plays a role in enhancing immune responses [42], IL-1β is known as a pro-inflammatory cytokine and plays a pivotal role in regulating immune response [43], IL-8 is a chemokine CXC ligand and play pivotal roles in mediating inflammatory responses to invading pathogens [44], heat shock protein (Hsp70) contributes to acquired thermal and oxidative tolerance [45]. ISG15 is an interferon-stimulated gene and involved in host immune defense against pathogen infections [46]. Hp is released from hepatocytes under inflammatory conditions to protect healthy cells from being damaged by pathogens or from self-destructive mechanisms [47], SAA is a major positive acute phase protein, playsing a vital role in the signaling processes and elimination of invading pathogens [48]. These findings suggested that SsHMGB2 play an important role in regulating the innate immune system. Similar result was observed in a study, in which tongue sole HMGB2 protein augmented the expression of several immune-related genes [26].

In conclusion, a HMGB2 homologue, SsHMGB2, was characterized and identified from black rockfish. The expression of SsHMGB2 could be induced by bacterial infection, and purified recombinant SsHMGB2 protein possesses DNA-binding ability and immunoregulatory effect. The results of our study also demonstrated that rSsHMGB2 exerted antibacterial effect under *in vivo* conditions. All these observations indicated that teleost HMGB2 is a functional DNA-binding cytokine, which possesses immunoregulatory property and plays a role in innate immunity against bacterial infection.

Acknowledgements

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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.2018.10.066.

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