Fish & Shellfish Immunology 36 (2014) 453–458

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Short communication

Molecular cloning and characterization of SOCS-2 from Manila clam *Ruditapes philippinarum*

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ARTICLE INFO

Article history:
Received 25 September 2013
Received in revised form
17 December 2013
Accepted 19 December 2013
Available online 27 December 2013

Keywords:
Manila clam
Ruditapes philippinarum
Suppressor of cytokine signaling (SOCS)
LPS
poly I:C

ABSTRACT

Suppressor of cytokine signaling (SOCS) family members are key regulators of immunological homeostasis. In this study, we have discovered the SOCS-2 member from Manila clam *Ruditapes philippinarum* and further analyzed its immune responses against lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (poly I:C). Amino acid sequence of RpSOCS-2 consists of cytokine inducible SRC homology 2 (SH2) and SOCS box domains similar to vertebrate SOCS counterparts. It has the highest amino acid identity (41%) with Pacific oyster (*Crassostrea gigas*) SOCS-2 and showed close evolutional relationship with disk abalone (*Haliotis discus discus*) SOCS-2. Tissue specific expression results showed that RpSOCS-2 was constitutively expressed in all examined tissues with the highest level in gill tissue of un-challenged clams. RpSOCS-2 mRNA expression was up-regulated by LPS and poly I:C challenge in gills. Discovery of RpSOCS-2 homologue and expression analysis would support for understanding evolutional relationships and their role in innate immune responses in mollusks, respectively.
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1. Introduction

Cytokines affect vast array of biological process and act as hormones of the immune system [1]. Most cytokines can bind to specific cell surface receptors which event initiates a cascade of downstream signals for regulating gene expression. It is mediated by transcription factors such as AP-1 and NF-kB [2]. Innate immune responses are functioning to defend against invading pathogens and lead to activate pro-inflammatory reactions. However, excessive inflammatory responses are harmful to host cells. Therefore, process of immune function has to be tightly controlled in order to maintain homeostasis during immune defense responses [3,4]. Over expression of cytokines are controlled by suppressing cytokine signals that are regulated by number of proteins including protein inhibitor of activated signal transducers and activators of transcription (PIAS) protein, tyrosine phosphatases, and SOCS family members [5]. In mammals, SOCS protein family consists of eight known members containing SOCS-1 to SOCS-7 and SH2 domain containing protein (CISH). All these members share a central SH2 and C-terminal SOCS box domains [5,6]. SOCS-2 genes have been identified from several fish species including *Oncorhynchus mykiss* [7], *Ctenopharyngodon idella* [ACV85622], *Danio rerio*, *Takifugu rubripes*, *Gasterosteus aculeatus*, and *Tetraodon nigroviridis*, and *Oryzias latipes* [8] but very few from invertebrates. Cytokine- like activities have been demonstrated in invertebrates such as starfish and *Drosophila*. Stec and Zeidler [9] have shown 3 SOCS-like proteins (SOCS16D, SOCS36E, and SOCS44A) in *Drosophila* genome which all share many similarities to their human homologues. As an example, SOCS36E, SOCS44A have been shown to interact with JAK/STAT (Janus kinase/signal transducers and activators of transcription) and EGFR (epidermal growth factor receptor) signaling pathways under positive and negative regulation which gives some evidence for similar role in invertebrates. In mollusks, only disk abalone (*Haliotis discus discus*) [3] SOCS-2 has

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http://dx.doi.org/10.1016/j.fsi.2013.12.012

been characterized and Pacific oyster (*Crassostrea gigas*) SOCS-2 (EKC24772) was identified [10]. In this study, we report the first bivalve SOCS-2 gene from marine mollusk of Manila clam *Ruditapes philippinarum*. We investigated the transcriptional regulation of RpSOCS-2 after challenge with LPS and poly I:C to give evidence of SOCS-2 role in regulation of immune system in clam.

2. Materials and methods

2.1. Identification and molecular characterization of RpSOCS-2

We identified a nucleotide sequence which matches with known SOCS-2 genes by screening of Manila clam transcriptome database [11]. It was named as *R. philippinarum* SOCS-2 (RpSOCS-2). Molecular characterization of RpSOCS-2 was done using several bioinformatics tools. Pair-wise and multiple sequence alignment of RpSOCS-2 were analyzed using ClustalW version 1.8 program [12]. The phylogenetic relationship of RpSOCS-2 was determined using the neighbor-joining (NJ) method and MEGA 3.1 program [13] with boot strapping values taken from 1000 replicates. SignalP program (http://www.cbs.dtu.dk/) was used to predict the presence of signal peptide sequence.

2.2. Experimental animals and immune challenge

Manila clams were collected from the Eastern coastal region of Jeju Island, Republic of Korea. Animals had average shell length of 2.5–3.0 cm and apparently healthy. Animals were kept in flat-bottomed fiberglass tanks (80 L) with aerated sand-filtered seawater. All animals were acclimatized for 1 week under laboratory conditions that maintained the seawater at 20 ± 1 °C and salinity of 32 ± 1‰. The maximum stock density was kept at 40 animals per tank during the experiment. Different tissues (adductor muscle, mantle, siphon, gill, and foot) and hemocytes were collected from five un-challenged Manila clams for evaluating mRNA expression profile. The clam hemolymph was collected from the posterior adductor muscle sinus using 1 mL syringe connected with 26-gauge needle. The hemocytes were obtained after centrifugation of hemolymph at 3000 rpm for 10 min at 4 °C. We injected two immune modulators namely LPS and poly I:C to investigate the immune response of RpSOCS-2 at transcriptional level. The LPS (*Escherichia coli* 0127:B8 Sigma, USA) and poly I:C (Sigma Aldrich; USA) were diluted into 1 mg/mL using 0.9% saline as stock solutions. Clams were intramuscularly injected with 100 μL of LPS (100 μg/clam) and poly I:C (100 μg/clam). A blank group was established as un-injected, while other control group was injected with 100 μL of 0.9% saline. Gill samples were taken from five animals at 3, 6, 12, 24, and 48 h post-challenge. All samples were immediately snap-frozen in liquid nitrogen and stored at −70 °C until further use.

2.3. Tissue specific expression and transcriptional regulation of RpSOCS-2

Total RNA was isolated from pooled gill tissues samples (200 mg) of five animals using the Qiazol lysis reagent (Qiagen, USA) and relevant standard procedures. First-strand cDNA synthesis was carried out using 1 μg of total RNA as template with the PrimeScript™ first strand synthesis kit (Takara, Japan) by following the manufacturer's instructions. The cDNA product was diluted by

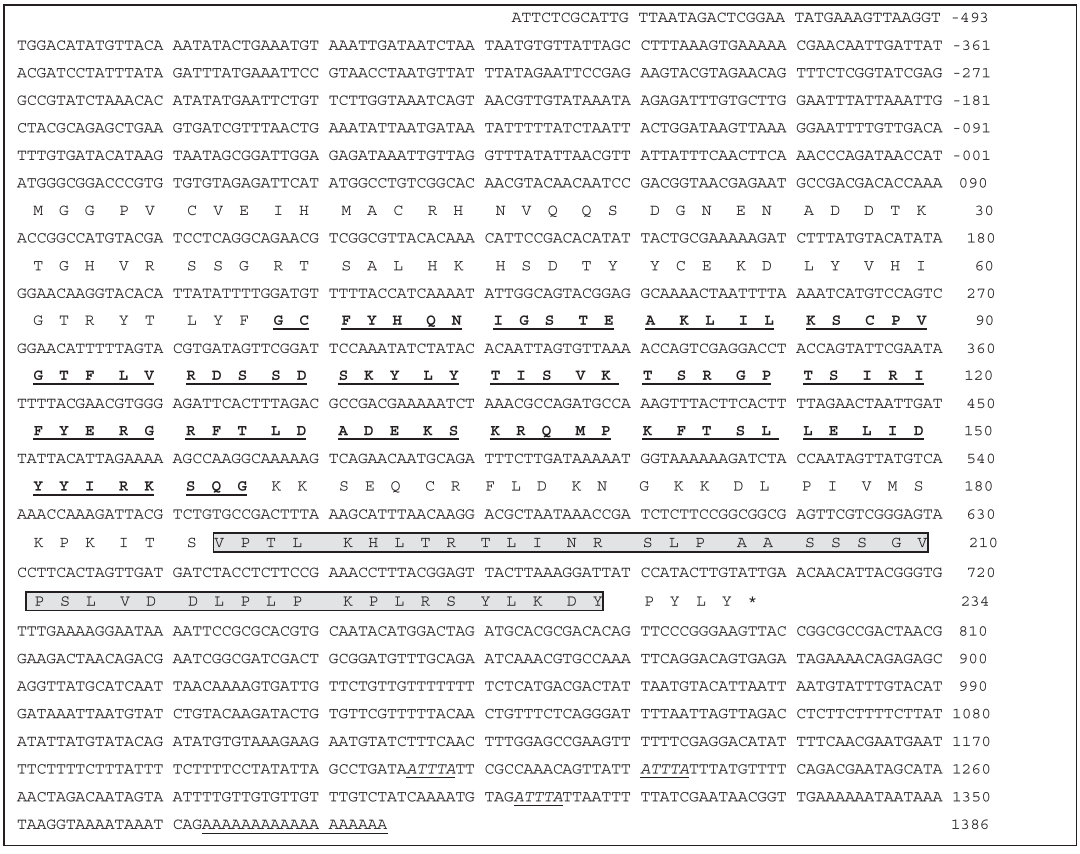

Fig. 1. The complete nucleotide and deduced amino acid sequences of the Manila clam SOCS-2 (RpSOCS-2) cDNA. The predicted SH2 domain and SOCS box are bold underlined and gray boxed, respectively. The poly (A) tail is underlined at the end of the nucleotide sequence.

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