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Short communication

Molluskan fasciclin-1 domain-containing protein: Molecular characterization and gene expression analysis of fasciclin 1-like protein from disk abalone (*Haliotis discus discus*)

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ABSTRACT

Cell-to-cell contacts play a key role in multicellular systems and organisms. Fasciclin-1 (FAS-1) is a lipid-linked membrane associated glycoprotein that is a member of a newly recognized family of cell adhesion molecules sharing features with the immunoglobulins, cadherins, integrins, and selectins. Here, we report the identification and molecular characterization of a novel FAS-1 domain-containing cDNA from disk abalone (*Haliotis discus discus*), including its gene expression profile and immune response to bacterial stimuli and tissue injuries. Designated as Abfac1, the 909 bp open reading frame (ORF) encodes 303 amino acid (aa) residues with a predicted molecular mass of 33 kDa and isoelectric (pI) value of 4.9. The aa sequence contains two FAS-1 domains and three conserved regions, FRa motif, H-box, and FRb motif. Phylogenetic analysis showed the closest relation to Jellyfish cell adhesion protein. In healthy abalone, Abfac1 expression is highest in hepatopancreas followed by mantle and lowest in digestive gland. In immune-stimulated abalones, relative Abfac1 mRNA expression was increased in hemocytes by ~11-fold at 48 h after the *Vibrio parahaemolyticus* infection, by 3.1-fold at 6 h after the *Listeria monocytogenes* infection and by ~9-fold at 6 h after the LPS injection. Similarly, tissue injuries caused significant increase of relative mRNA expression by 3.5-fold in hemocytes and by ~10-fold in mantle at 12 h post-injury. These results suggest that the novel member of the FAS-1 domain-containing protein family, Abfac1, may be involved in immune response and cell adhesion in disk abalone.

1. Introduction

In multicellular systems and organisms, cell-to-cell contacts play a key role in development, physiology, and morphogenesis. The fasciclins are a newly recognized family of cell adhesion molecules that share features of the immunoglobulins, cadherins, integrins, and selectins (Huber and Sumper, 1994). Fasciclin-1 (FAS-1) is a lipid-linked membrane associated glycoprotein that was originally identified by a monoclonal antibody (mAb) screen of embryo tissues from the grasshopper *Schistocerca americana* (Bastiani et al., 1987: Elkins et al., 1990; Huber and Sumper, 1994). In these insects, FAS-1 protein was found to be expressed on a subset of fasciculating axons during neuronal development, where it mediated axon guidance, the process by which axons extend from the neural cell body to reach other target neurons or muscles cells (Bastiani et al., 1987). Subsequent studies of FAS-1 homologues revealed that they are loosely conserved between species, having approximately 25% amino acid (aa) identity and 50% similarity (Reynolds et al., 2000). All FAS-1 proteins consist of at least one FAS-1 domain (130–150 aa in length) and three conserved regions: the FRa motif, the H-box, and the FRb motif (Hu et al., 1998; Zinn et al., 1988). The number of FAS-1 domains varies according to species, with the proteins described to date containing one, two or four repeating homologous FAS-1 domains (Hu et al., 1998; Sato et al., 2004). In addition, the Fas-1 gene family has been shown to be phylogenetically diverse. FAS-1 domain-containing cell adhesion proteins have been identified in secreted and membrane anchored proteins from several species,

Abbreviations: FAS-1, fasciclin-1; ORF, open reading frame; aa, amino acid; Abfac1, disk abalone fasciclin-1 domain containing gene/protein; mRNA, messenger ribonucleic acid; mAb, monoclonal antibody; EST, expressed sequence tag; LPS, lipopolysaccharide; i.m., intramuscular injection; p.i., post infection/induction; cDNA, complementary deoxyribonucleic acid; qPCR, quantitative real-time polymerase chain reaction; bp, base pair; UTR, un-translated region; WSSV, white spot syndrome virus; ECM, extracellular matrix; HC, hemocyte; Gl, gill; Mn, mantle; Ms, muscle; Dg, digestive gland; Hp, hepatopancreas.

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including *Synechocystis* sp. (MPB70) (Ulstrup et al., 1995), *Volvox* (algal-CAM) (Huber and Sumper, 1994), Arabidopsis (FLAs) (Johnson et al., 2003), lung fluke (Pwfas-I) (Song et al., 2010b), Drosophila (Fas I and Mfas) (Elkins et al., 1990; McAllister et al., 1992), sea urchin (EBP-α) (Sato et al., 2004), fish (βig-h3) (Hirate et al., 2003), chicken (RGD-CAP) (Kawamoto et al., 1998), mouse (*βigH3*) (Lindsley et al., 2005), and human (OSF-2) (Adachi and Tsujimoto, 2002). The evolutionary conservation of the fasciclin-1 protein family suggests that FAS-1 proteins may play key roles in some shared physiological process(es).

The temporal and spatial expression patterns of Fas-1 genes have been studied in some of these species. In Drosophila, Fas-1 was found to be expressed in a complex pattern throughout embryonic development (McAllister et al., 1992). In mice, multiple fasciclin-containing adhesion molecules were found to be expressed on endocardial cushions undergoing the key steps of heart development, including seeding, proliferation, differentiation, fusion, mesenchymal condensation, and remolding (Lindsley et al., 2005). The mammalian-based studies have identified a total of four fasciclin-containing genes to date, and these are periostin, βig-H3, stabilin-1, and stabilin-2. Both periostin and βig-H3 have been characterized as secreted proteins induced by transforming growth factor β signaling; moreover, βig-H3 is known to contribute to corneal epithelial cell adhesion through interaction with $\alpha_3\beta_1$ integrin (Ferguson et al., 2003; Kim et al., 2000). Thus, the collective research on FAS-1 domain-containing protein homologues has suggested that these proteins may serve important but distinct functions in the different species.

Here, we report the characterization of a novel FAS-1 domain-containing protein in disk abalone (*Haliotis discus discus*). This newly recognized Abfac1 protein represents the first molluskan FAS-1 domain-containing protein described to date and suggests a putative functional role for FAS-1 in the immune system.

2. Materials and methods

2.1. Experimental animals

Live disk abalones were obtained from the Youngsoo abalone farm (Jeju Island, Republic of Korea) and they were maintained at Marine and Environmental Research Institute at Jeju National University. Upon transfer to the laboratory, the animals were housed in flat-bottomed fiberglass tanks (250 L) filled with aerated seawater (30‰ salinity) at 18 ± 1 °C and fed with a daily diet of fresh seaweed (*Undaria pinnatifida*). Abalones were acclimatized for seven days prior to experimentation.

2.2. Disk abalone cDNA library construction and isolation of Abfac1 cDNA

A normalized cDNA library of disk abalone was constructed by isolating mRNA from healthy disk abalones. The basic procedures of cDNA library construction, normalization, and initial sequencing were carried out as previously described (De Zoysa et al., 2008). After sequencing the 5′-ends of 6700 clones with a specific primer to the cloning vector, a single putative clone (in the expressed sequence tag (EST) database) that showed similarity to known FAS-1 domain-containing proteins was identified by NCBI BLAST analysis (http://www.ncbi.nlm.nih.gov/blast/). The complete cDNA sequence of Abfac1 was deposited in GenBank under the accession number GQ903764.

2.3. Sequence characterization and phylogenetic analysis of Abfac1

The nucleotide and aa sequences of the disk abalone FAS-1 domain-containing gene (Abfac1) were compared with the NCBI nucleotide and protein databases by using the respective BLASTX and BLASTP programs. To determine the conserved domains and active sites in Abfac1, the motif scan (http://hits.isb-sib.ch/cgi-bin/p/PFSCAN) and PROSITE (http://www.expasy.ch/prosite/) algorithms were used, respectively. The identity and similarity percentages at the aa level were detected using the EMBOSS pairwise alignment algorithm (http://www.ebi.ac.uk/Tools/emboss/align/). Phylogenetic analysis was performed by the MEGA (molecular evolutionary genetic analysis) software package using the Neighbor-Joining method (version 5.05; http://megasoftware.net) with bootstrap values taken from 1000 replicates.

2.4. Tissue distribution analysis and immune challenge experiment

Samples of gills, mantle, muscle, hemocyte, hepatopancreas, and digestive gland were isolated from three healthy abalones. The hemocytes were isolated from hemolymph that had collected from the pericardial cavity via sterilized syringe and immediately centrifuged at 3000 ×*g* for 10 min at 4 °C. To determine the immune responsiveness of Abfac1 gene expression, immune stimulation/challenge experiments were carried out with a well-known endotoxin, lipopolysaccharide (LPS) which is a cell wall component of the Gram-negative bacteria, and two species of pathogenic bacteria known to infect disk abalones. Two bacterial strains were obtained from the Korean Collection for Type Cultures (KCTC): a Gram-negative strain, *Vibrio parahemolyticus* (KCTC2729); and a Gram-positive strain, *Listeria monocytogenes* (KCTC3710). *V. parahemolyticus* was cultured on Luria–Bertani (LB) marine agar plates at 25 °C overnight. A single colony was selected from the plate to inoculate 4 mL of LB marine broth and grown at 25 °C for 16 h with shaking at 200 rpm. Similarly, *L. monocytogenes* was cultured on an LB agar plate at 30 °C overnight and inoculated in LB broth at 30 °C for 16 h. Then, both bacterial cultures (1.5 mL each) were respectively centrifuged at 7000 ×*g* at 4 °C for 5 min. The supernatant was discarded and the bacterial pellets were re-suspended in saline (0.9% NaCl) to have 1×10^5 CFU/mL. Experimental groups of abalones were intramuscularly injected (i.m.) with 100 μL of the bacterial suspensions accounting for 1×10^4 CFU per abalone, respectively. Similarly, another group of abalones was injected (i.m.) with 100 μL (500 μg per abalone) of LPS (*Escherichia coli*; 055:B5; Sigma-Aldrich, USA) suspended in saline. A control group of abalones was injected with 100 μL (per abalone) of saline as positive control and another group of abalones were maintained as a blank/un-injected control. Abalones from the experimental and control groups were sacrificed at 3, 6, 12, 24 and 48 h post-infection/induction (p.i.) to immediately harvest tissue samples. A minimum of four abalones was sacrificed at each time point for each challenge. The tissue samples were snap-frozen in liquid nitrogen and stored at −80 °C until processing for RNA isolation.

A tissue injury experiment was carried out by experimentally damaging the shell and injuring the mantle of healthy abalones. Hemocyte and mantle tissue samples were isolated from three abalones at 3, 6, 9, 12, 24, 48 and 120 h post-injury. The isolated tissues were stored at −80 °C. Non-injured abalones served as the control.

2.5. RNA extraction and cDNA synthesis

Total RNA was extracted from the isolated tissues (50 mg from each of the triplicate replicates) using the Tri Reagent™ (Sigma, USA), in accordance with the manufacturer's protocol. The RNA concentration was determined by measuring the absorbance at 260 nm and diluted to a concentration of 1 μg/μL. Aliquots of 2.5 μg RNA were applied as template to cDNA synthesis using SuperScript III First-strand cDNA Synthesis Kit (Invitrogen, USA). Briefly, RNA was incubated with 1 μL of 50 μM oligo $(dT)_{20}$ and 1 μL of 10 mM dNTP at 65 °C for 5 min. Then, 4 μL of 10× cDNA synthesis buffer, 2 μL of dithiothreitol (DTT; 0.1 M), 1 μL of RNaseOUT™ (40 U/μL), and 1 μL of Superscript III reverse transcriptase (200 U/μL) were added and the solution incubated for 1 h at 45 °C. The reaction was terminated by adjusting the temperature to 85 °C for 5 min. Finally, 1 μL of