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Short communication

A homolog of teleostean signal transducer and activator of transcription 3 (STAT3) from rock bream, *Oplegnathus fasciatus*: Structural insights, transcriptional modulation, and subcellular localization

S.D.N.K. Bathige[a,b], William Shanthakumar Thulasitha[a,b], Navaneethaiyer Umasuthan[a,b], J.D.H.E. Jayasinghe[a,b], Qiang Wan[b], Bo-Hye Nam[c], Jehee Lee[a,b,*]

[a] Department of Marine Life Sciences, School of Marine Biomedical Sciences, Jeju National University, Jeju Self-Governing Province, 63243, Republic of Korea
[b] Fish Vaccine Research Center, Jeju National University, Jeju Special Self-Governing Province 63243, Republic of Korea
[c] Biotechnology Research Division, National Institute of Fisheries science, 408-1 Sirang-ri, Gijang-up, Gijang-gun, Busan 46083, Republic of Korea

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ABSTRACT

Signal transducer and activator of transcription 3 (STAT3) is one of the crucial transcription factors in the Janus kinase (JAK)/STAT signaling pathway, and it was previously considered as acute phase response factor. A number of interleukins (ILs) such as IL-5, IL-6, IL-9, IL-10, IL-12, and IL-22 are known to be involved in activation of STAT3. In addition, various growth factors and pathogenic or oxidative stresses mediate the activation of a wide range of functions via STAT3. In this study, a STAT3 homolog was identified and functionally characterized from rock bream (RbSTAT3), *Oplegnathus fasciatus*. *In silico* characterization revealed that the RbSTAT3 amino acid sequence shares highly conserved common domain architectural features including N-terminal domain, coiled coil domain, DNA binding domain, linker domain, and Src homology 2 (SH2) domains. In addition, a fairly conserved transcriptional activation domain (TAD) was located at the C-terminus. Comparison of RbSTAT3 with other counterparts revealed higher identities (>90%) with fish orthologs. The genomic sequence of *RbSTAT3* was obtained from a bacterial artificial chromosome (BAC) library, and was identified as a multi-exonic gene (24 exons), as found in other vertebrates. Genomic structural comparison and phylogenetic studies have showed that the evolutionary routes of teleostean and non-teleostean vertebrates were distinct. Quantitative real time PCR (qPCR) analysis revealed that the spatial distribution of *RbSTAT3* mRNA expression was ubiquitous and highly detectable in blood, heart, and liver tissues. Transcriptional modulation of *RbSTAT3* was examined in blood and liver tissues after challenges with bacteria (*Edwardsiella tarda* and *Streptococcus iniae*), rock bream irido virus (RBIV), and immune stimulants (LPS and poly (I:C)). Significant changes in *RbSTAT3* transcription were also observed in response to tissue injury. In addition, the transcriptional up-regulation of *RbSTAT3* was detected in rock bream heart cells upon recombinant rock bream IL-10 (rRbIL-10) treatment. Subcellular localization and nuclear translocation of rock bream STAT3 following poly (I:C) treatment were also demonstrated. Taken together, the results of the current study provide important evidence for potential roles of rock bream STAT3 in the immune system and wound healing processes.

1. Introduction

Signal transducer and activator of transcription (STAT) proteins play a vital role in biological systems by activating the expression of specific genes or set of genes in response to a wide variety of internal and external stimuli. Signals from cytokines, growth factors, or hormones are transduced following the dimerization of phosphorylated STATs and subsequent translocation into the nucleus, where they bind DNA response elements of target genes (Ihle, 1996). Seven STATs including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 have been identified and well characterized in mammals (Stark and Darnell, 2012). All these STATs share common structural features including six conserved domains with different functions:

* Corresponding author at: Marine Molecular Genetics Lab, Department of Marine Life Sciences, College of Ocean Science, Jeju National University, 66 Jejudaehakno, Ara-Dong, Jeju, 63243, Republic of Korea.
E-mail addresses: jeheedaum@hanmail.net, jehee@jejunu.ac.kr (J. Lee).

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the N-terminal domain regulates nuclear translocation (Strehlow and Schindler, 1998), the coiled coil domain interacts with various proteins, the DNA binding domain interacts with DNA, the Src homology 2 (SH2) domain plays a vital role in signal transduction via phosphotyrosine, the linker domain connects the DNA binding domain and SH2 domain, and the transcriptional activation domain (located at the C-terminal) regulates transcriptional responses (Kisseleva et al., 2002).

STAT3 was independently identified in 1994 by two different groups. Initially, it was considered as acute phase response factor activated by interleukin-6 (IL-6) (Akira et al., 1994) or a member of the STAT family activated by epidermal growth factor and IL-6 (Zhong et al., 1994). In addition, Janus kinase 2 (JAK2), one member of JAK family, was identified as a major mediator of STAT3 phosphorylation (Aggarwal et al., 2006). Later studies have shown that signal transduction of mammalian STAT3 is initiated by a number of growth factors and cytokines such as growth hormone (Gronowski et al., 1995), IL-5 (Stout et al., 2004), IL-9 (Demoulin et al., 1996), IL-10 (Wehinger et al., 1996), IL-12 (Jacobson et al., 1995), IL-22 (Radaeva et al., 2004), and leptin (Vaisse et al., 1996). Apart from that, other factors activating STAT3 include oxidative stress (Carballo et al., 1999), ultraviolet B (Ahsan et al., 2005), osmotic shock (Gatsios et al., 1998), and viral infection (Yoshida et al., 2002). STAT3 is known to be the essential transcription factor mediating pleiotropic activities including immune responses, cell proliferation, inflammation, embryo development, brain development, initiation of gene expression, and apoptosis (Alonzi et al., 2001; Di Domenico et al., 2010; Levy and Lee, 2002; Takeda et al., 1998; Takeda et al., 1997; Yang et al., 2007). Thus far, very little information has been reported on fish STAT3, even though studies describing the immunobiological activities of a few fish species, *Siniperca chuatsi* (Guo et al., 2009), *Scophthalmus maximus* (Wang et al., 2011b), and *Epinephelus* spp. (Huang et al., 2014), have demonstrated a possible role of STAT3 against viral/bacterial infections. A previous study of *Danio rerio* reported the involvement of STAT3 in regulation of cell migration during gastrulation (Yamashita et al., 2002), and one recent study has shown the association of STAT3 with different forms of programmed cell death (Huang et al., 2015).

Rock bream (*Oplegnathus fasciatus*) is an economically important fish species in South Korean aquaculture. In recent decades, a great loss of fish production in the aquaculture industry has occurred due to the threat of viral and bacterial diseases (Park, 2009). Rock bream iridovirus (RBIV) causes the most well-known viral disease, leading to high mortality of rock bream in South Korea (Jung and Oh, 2000; Li et al., 2011). Edwardsiellosis and streptococcosis caused by *Edwardsiella tarda* and *Streptococcus iniae*, respectively are bacterial diseases identified in aquaculture industries (Park et al., 2012; Sun et al., 2011). Therefore, understanding the immune defense mechanisms that counter these microbial infections and other virulent factors is crucial in establishing a sustainable rock bream industry.

In the current study, an ortholog of STAT3 identified from rock bream (RbSTAT3) was characterized at the cDNA and genomic DNA levels. The spatial mRNA expression profile in different tissues and the temporal mRNA expressions upon *in vivo* and *in vitro* challenges were also assessed. In addition, the mediation of RbSTAT3 on the wound healing process was investigated by analyzing the transcriptional profile following tissue injury. Finally, the subcellular localization of RbSTAT3 under a normal physiological condition and an immune stress condition was examined.

2. Material and methods

2.1. Identification of cDNA and genomic sequences

A rock bream transcriptome database was constructed using a Roche 454 Genome sequencer FLX (GS-FLX^{TM}) platform, a next generation sequencing (NGS) technology (Droege and Hill, 2008). Contigs in the sequence database were generated from shotgun sequencing reads using *de novo* assembling technique. A contig with a full-length coding sequence (CDS) with higher homology to known STAT3 counterparts was identified using the NCBI Basic Local Alignment Search Tool (BLAST) algorithm.

The genomic sequence of *RbSTAT3* was obtained from a rock bream bacterial artificial chromosome (BAC) library. The BAC library was custom constructed (Lucigen®, USA) using genomic DNA (gDNA) isolated from rock bream blood cells. Briefly, randomly sheared gDNA segments (>100 kb) were cloned into a pSMART® BAC vector, and 92,160 independent clones were obtained. Each clone was arrayed in 240 384-deep well plates, and plasmids were systematically arranged into 20 super pools with each super pool comprised of 12 plate pools, 16 row pools, and 24 column pools. The relevant clone bearing *RbSTAT3* was localized in the BAC library by means of a PCR based technique with gene specific primers (Supplementary Table 1) based on the manufacturer's protocol. The positive BAC clone was isolated and purified using the QIAGEN® Plasmid Midi Kit (Germany), and the genomic sequence of *RbSTAT3* was obtained via GS-FLX 454 pyrosequencer (Macrogen, South Korea).

2.2. In silico *analysis*

The open reading frame (ORF) and amino acid (aa) sequence of RbSTAT3 were analyzed using DNAssist software (version 2.2) and BLAST algorithm using the NCBI web tool with default parameters (http://www.ncbi.nlm.nih.gov/blast). The Expert Protein Analysis System (http://expasy.org/tools/) was employed to determine the characteristic domain architectural features of RbSTAT3. The aa sequence of STAT3 and other orthologs obtained from GenBank at NCBI were aligned using the ClustalW multiple alignment program. The Neighbor-Joining (NJ) phylogenetic tree was constructed with the MEGA 5.05 program based on the amino acid sequences from different taxonomic groups. Bootstrap analysis with 5000 replicates was executed to assess the branch strength of the phylogenetic tree. Tertiary model structure of RbSTAT3 was generated by submitting the aa sequence to the SWISS-MODEL, which is an automated protein structure homology modeling server (Arnold et al., 2006). Crystal structure of mouse STAT3 (PDB: 1BG1) with a resolution of 2.2 Å was utilized as a template, and a 3D surface diagram was visualized by using PyMOL molecular graphic software version 1.3 (DeLano, 2002). The boundaries of the exons and introns of the *RbSTAT3* genomic sequence were predicted by the Spidey tool at NCBI based on the alignment of cDNA and the genomic sequence. The possible transcription factor binding sites (TFBS) at the 5′-flanking region of *RbSTAT3* genomic sequence were predicted by the AliBaba 2.1 online prediction tool.

2.3. Experimental animals, in vivo *immune and injury challenge experiments, and tissue collection*

The rock breams, *O. fasciatus* (~50 g in body weight) were obtained from the Ocean and Fisheries Research Institute in Jeju Special Self-Governing Province, Republic of Korea. Fish were