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Identification, phylogeny and expression analysis of suppressors of cytokine signaling in channel catfish

Jun Yao[a,1], Weijie Mu[a,b,1], Shikai Liu[a], Jiaren Zhang[a], Haishen Wen[b], Zhanjiang Liu[a,*]

[a] The Fish Molecular Genetics and Biotechnology Laboratory, Aquatic Genomics Unit, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn, AL 36849, USA
[b] Fisheries College, Ocean University of China, 5 Yushan Road, Qingdao 266003, Shandong, PR China

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ABSTRACT

The suppressors of cytokine signaling (SOCS) family genes play important roles in regulating a variety of signal transduction pathways that are involved in immunity, growth and development. Because of their importance, they have been extensively studied in mammalian species, but they have not been systematically studied among teleost fish species. In this study, a total of 12 SOCS genes were characterized to understand the molecular mechanisms of SOCS function in channel catfish. Phylogenetic analyses suggested that all SOCS were clustered into two main clusters. Further syntenic analysis confirmed the phylogenetic analyses and allowed the annotation of SOCS genes in channel catfish. This work, for the first time, determined the expression profiles of the 12 SOCS genes after bacterial infections with *Flavobacterium columnare* and *Edwardsiella ictaluri* in channel catfish. The *SOCS1a* and *SOCS3a* were significantly up-regulated at 4 h after *F. columnare* challenge in the gill, but were down-regulated at later stages of pathogenesis. Similarly, *SOCS1a* and *CISH* were significantly up-regulated at 3 h in intestine under *E. ictaluri* infection, but were down-regulated at later stages of pathogenesis at 24 h and 3 days after infection. These expression patterns may indicate that SOCS genes could be induced in acute immune responses after bacterial infections, but the massive cytokine expression, especially chemokine expression after the first day of infection may have had negative feedback leading to the overall down-regulation of the expression of SOCS genes. Moreover, the differential expression patterns of SOCS genes in the catfish gill and intestine after *F. columnare and E. ictaluri* infection demonstrated that the regulation of SOCS gene expression was both tissue-specific and time-dependent. Taken together, these results suggested that SOCS genes were involved in immune responses to bacterial invasions, and these results set the foundation for future studies of SOCS gene functions.

1. Introduction

Cytokines play critical roles in vertebrate homeostasis and immune regulation by activating cell surface receptor complexes at the cell membrane, setting off a cascade of processes that leads to induction, enhancement or inhibition of a number of cytokine-regulated genes in the nucleus (Ilangumaran et al., 2004; Jin et al., 2008; Rico-Bautista et al., 2006). Most cytokine receptors transduce signals by interacting with the members of Janus kinase (JAK) family that are non-covalently bound to membrane proximal regions of receptor chains (Heim, 1999; Zhang et al., 2010). Activated JAKs, in turn, phosphorylate signal transducer and activator of transcription (STAT) proteins (Dalpke et al., 2008; Wang and Secombes, 2008). The dimers of phosphorylated STATs subsequently translocate to the nucleus and activate gene expression (Alexander, 2002; Dalpke et al., 2008; Zhong et al., 2005). In order to prevent the disorder of initiation, duration, and magnitude of cytokine signaling, maintain the normal homeostasis and cellular functions, the cytokine signaling is negatively regulated by a number of proteins. One of the most important feedback inhibitors of cytokine receptor signaling is the suppressor of cytokine signaling (SOCS) family (Alexander and Hilton, 2004; Haque et al., 2000; Yasukawa et al., 2000).

It is known that all SOCS protein family members possess a central SH2 domain, and a conserved C-terminal motif named as the SOCS box (Endo et al., 1997; Hilton et al., 1998; Naka et al., 1997; Starr et al., 1997). However, the amino-termini of these proteins exhibit a high level of variation in both lengths and amino acid sequences (Dalpke et al., 2008). SOCS1 and SOCS3 contain an additional N-terminal domain in SH2 region named kinase inhibitory

* Corresponding author. Tel.: +1 334 844 4054; fax: +1 334 844 9208.
E-mail address: liuzhan@auburn.edu (Z. Liu).
[1] These authors contributed equally.

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region (KIR), which acts as a pseudo-substrate for JAKs (Yasukawa et al., 1999). Moreover, SOCS2 and CISH possess an N-terminal extended SH2 subdomain (N-ESS) instead of KIR (Bullock et al., 2006). It is suggested that SOCS proteins inhibit signal transduction of type I and II cytokine receptors, which is achieved primarily by acting at the level of activated receptors and JAKs (Dalpke et al., 2008). All SOCS proteins bind to phosphorylated tyrosine residues through their SH2 domains (Krebs and Hilton, 2001).

The SOCS family is well documented in mammals. However, studies in non-mammalian species are limited (Jin et al., 2007; Kuliyev et al., 2005). The numbers of SOCS genes vary among species. In teleost fish, CISH and SOCS1-7 molecules were initially found in several fish species, including *Tetraodon nigroviridis*, *Danio rerio*, *Fugu rubripes*, *Gasterosteus aculeatus*, *Oncorhynchus mykiss*, and *Cyprinus carpio* (Jin et al., 2007; Wang et al., 2010; Wang and Secombes, 2008; Xiao et al., 2010). Copy numbers of SOCS family genes are generally higher in fish genomes than in mammalian genomes (Jin et al., 2008).

Channel catfish (*Ictalurus punctatus*) is the most important aquaculture species in the United States. Numerous studies related to immune genes have been conducted, including chemokines (Bao et al., 2006; Peatman et al., 2006; Peatman and Liu, 2007), antimicrobial peptides (Bao et al., 2006; Wang et al., 2006; Xu et al., 2005), lectins (Takano et al., 2008), and pattern-recognition receptors (PRRs) such as toll-like receptors (TLRs, Baoprasertkul et al., 2006, 2007a,b; Bilodeau and Waldbieser, 2005; Zhang et al., 2013), NOD-like receptors (NLRs, Sha et al., 2009; Rajendran et al., 2012a), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs, Rajendran et al., 2012b), and peptidoglycan receptors (Sun et al., 2014). However, systematic analysis of SOCS genes has not been conducted. The objectives of this study are to identify and annotate SOCS genes in channel catfish, and determine their expression profiles after bacterial infections.

2. Materials and methods

2.1. Database mining and gene identification

To identify the SOCS genes, the catfish RNA-Seq databases (Liu et al., 2011, 2012) and the whole genome database of channel catfish (unpublished data) were searched using available SOCS from teleost fish (*D. rerio, Ctenopharyngodon idella, T. nigroviridis, G. aculeatus, Takifugu rubripes, Oryzias latipes*), bird (*Gallus gallus*), Amphibian (*Xenopus tropicalis*) and mammals (*Homo sapiens*, *Sus scrofa* and *Mus musculus*) as queries. The ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to predict the open reading frames of retrieved sequences. GENSCAN (Burge and Karlin, 1997) and FGENESH (Solovyev et al., 2006) were used to predict genes from genomic sequences. The predicted ORFs were verified by BLASTP against NCBI non-redundant protein database. Simple Modular Architecture Research Tool (SMART http://smart.embl-heidelberg.de) was used to predict the conserved domains based on sequence homology.

2.2. Phylogenetic analysis

SOCS genes identified from channel catfish together with SOCS genes from other species including human, mouse, chicken, frog and several other teleost fish were used to conduct the phylogenetic analysis. Multiple alignments of protein sequence was performed using ClustalW (Thompson et al., 2002) with default parameters. The maximum likelihood method was used to conduct phylogenetic analysis using MEGA 5 (Tamura et al., 2011). Jones–Taylor–Thornton (JTT) and gamma distributed rate with invariant sites (G + I) model were chosen based on the alignment result (Darriba et al., 2011). Gaps were removed by pair-wise deletion and 1000 bootstrap replicates were performed in phylogenetic analysis.

2.3. Syntenic analysis

To provide additional evidence of orthologies for the annotation, the syntenic analyses of SOCS members were conducted. Human and zebrafish were used for syntenic analysis. The deduced SOCS amino acids were used as queries to search against the draft catfish genome sequence database, and the genomic scaffolds containing the catfish SOCS genes were retrieved. FGENESH program (Solovyev et al., 2006) was used to identify neighboring genes of the catfish SOCS from the channel catfish genome scaffolds. Ensembl database and Genomicus (Louis et al., 2013) were utilized to get the conserved syntenic blocks of these genes among human, zebrafish and catfish.

2.4. Expression analysis of SOCS genes in healthy channel catfish tissues

To determine the expression of SOCS genes in healthy tissues, the high quality reads from the RNA-Seq dataset of doubled haploid channel catfish (Liu et al., 2012) were used for the analysis. The dataset was obtained using RNA from 19 tissues including head kidney, fin, pancreas, spleen, gill, brain, trunk kidney, adipose tissue, liver, stomach, gall bladder, ovary, intestine, thymus, skin, eye, swim bladder, muscle, and heart (Liu et al., 2012). RNA-Seq reads were first mapped to the reference sequences of all assembled contigs including 12 SOCS genes using CLC Genomics Workbench software package. The parameters for mapping were set as 95% or greater sequence identity with a maximum of two mismatches. The number of total mapped reads on each transcript was determined, and reads per kilobase exon sequences per million mapped reads (RPKM) from each of the 12 SOCS genes were obtained.

2.5. Expression analysis of SOCS following F. columnare and E. ictaluri infections

To determine the expression profiles of SOCS genes after bacterial infections, meta-analysis of RNA-Seq data was conducted with CLC Genomics Workbench software package. Briefly, the RNA-Seq datasets generated from the intestine of catfish after *E. ictaluri* infection (Li et al., 2012), and the gill of catfish after *F. columnare* infection (Sun et al., 2012) were used. The samples used to generate RNA-Seq data after *E. ictaluri* infection were collected at 3-h, 24-h and 3-day after infection, while the samples used to generate RNA-Seq data after *F. columnare* infection were collected at 4-h, 24-h and 48-h after infection. Because of using meta-analysis, samples from the same time points after infection of both diseases were not available. For mapping of the RNA-Seq reads, all the assembled transcripts were used as reference sequences. The reads from the SOCS transcripts were first mapped to their assembled contigs using mapping parameters of at least 95% sequence identity with a maximum of two mismatches being allowed. The number of total mapped reads on each transcript was determined, and RPKM were calculated. After normalization of the RPKM, fold changes were calculated using proportions-based Kal's test to determine the differential expression pattern between control and treatment groups with p-value <0.05. Transcripts with a minimal number of 5 reads were included for further analysis and those with expression fold change values of greater than 2 as differentially expressed genes.