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

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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

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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.

Table 1
Characteristics of channel catfish (*Ictalurus punctatus*) SOCS genes.

Gene name	mRNA size (bp)	5′-UTR (bp)	3′-UTR (bp)	Amino acids	Accession no.
SOCS1a	1095	270	267	186	JT412050
SOCS1b	1194	245	326	195	JT412991
SOCS3a	1285	180	462	201	NM_001201254
SOCS3b	3342	563	2110	223	JT461874
SOCS4	1167	0	6	387	KM387313
SOCS5a1	5930	301	4069	520	JT407405
SOCS5a2	5957	720	3542	565	JT412555
SOCS5b	3805	277	1836	544	JT414980
SOCS6a	6769	439	4749	527	JT406112
SOCS6b	5197	260	3425	504	JT409667
SOCS7	3157	315	529	771	JT414025
CISH	2607	169	1766	224	JT412537

3. Results

3.1. Identification of channel catfish SOCS genes

A total of 12 SOCS genes including SOCS1a, SOCS1b, SOCS3a, SOCS3b, SOCS4, SOCS5a1, SOCS5a2, SOCS5b, SOCS6a, SOCS6b, SOCS7 and CISH were identified in the channel catfish genome. Their characteristics including the open reading frames, 5′- and 3′-untranslated regions, the lengths of amino acid sequences of each protein, and their GenBank accession numbers, are summarized in Table 1. The SOCS1, SOCS3 and SOCS6 each has two copies, whereas SOCS5 has three copies (see below). SOCS2 was not identified in the channel catfish genome. All members of the catfish SOCS possessed the conserved functional domains: a central SH2 domain, and a conserved SOCS box (Fig. 1).

3.2. Phylogenetic analysis of channel catfish SOCS genes

To understand evolutionary relationships among different SOCS genes and provide further support for their annotation, phylogenetic analysis was constructed. As shown in Fig. 2, two main clades were observed, one clade included the SOCS1 and SOCS3, and the other clade included CISH/SOCS8, SOCS2, SOCS4, SOCS5/SOCS9, SOCS6, and SOCS7. Apparently, the channel catfish CISH, SOCS1b, SOCS3b, SOCS5a1, SOCS5a2, SOCS6b and SOCS7 were clustered with their counterparts from other species. However, the phylogenetic analysis of SOCS1a, SOCS3a, SOCS4, SOCS5b and SOCS6a did not provide strong support for the evolutionary relationship with their counterparts from other teleost fish, and therefore, these genes could not be annotated based on phylogenetic analysis alone.

3.3. Syntenic analysis of channel catfish SOCS genes

Syntenic analysis was conducted to provide additional evidence for orthologies of SOCS genes. Conserved sytenic blocks were first identified for all SOCS genes from human, zebrafish and channel catfish. For SOCS1b, SOCS3b, SOCS5a1, SOCS5a2, SOCS6b, SOCS7 and CISH, syntenic analysis provided additional evidence of orthologies, confirming the results from phylogenetic analysis (Fig. 3A–E, Supplementary Fig. S1). For SOCS1a, SOCS3a, SOCS4, SOCS5b, SOCS6a, where phylogenetic analysis alone could not provide sufficient evidence for orthologies, syntenic analysis provided clear orthologies for all of them (Fig. 3A–E). For instance, the syntenic region was well conserved between the catfish and zebrafish genomic regions containing the SOCS1a gene (Fig. 3A); similarly, conserved syntenic blocks were identified for SOCS3a (Fig. 3B), SOCS4 (Fig. 3C), SOCS5b (Fig. 3D), and SOCS6a (Fig. 3E). As shown in Fig. 3D, SOCS5a1 in catfish and zebrafish, as well as SOCS5 in human, shared the same neighboring genes of msh2 and pigf. SOCS5a2 in catfish and zebrafish had the neighboring genes of prkceb, mcfd2 and ttc7a. In addition, catfish SOCS5b was located between sid1 and pmsa6l in the genomes, which was highly conserved with that of zebrafish. Meanwhile, the catfish SOCS5a2 shared the same neighboring gene calm with zebrafish SOCS5a1, SOCS5a2, SOCS5b, and human SOCS5, while catfish SOCS5b shared the same neighboring gene ppm1 with zebrafish SOCS5a1, SOCS5a2, SOCS5b and human SOCS5, suggesting that catfish SOCS5 molecules are orthologies with tetrapods SOCS5. Taken together, the syntenic analysis provided further evidence for supporting the annotation and nomenclature of SOCS genes in channel catfish, particularly for SOCS5.

3.4. Copy numbers of SOCS genes in catfish

In order to gain insights into SOCS gene evolution, the gene copy numbers of SOCS were also determined for several other representative vertebrates by data mining of public databases. As shown in Table 2, it's apparent that almost all the orthologs of SOCS genes in human, mouse, chicken, and several teleost species were found in the channel catfish genome (Table 2), with the only exception of SOCS2. Among these SOCS genes found in catfish, four catfish SOCS genes were duplicated: the SOCS1, SOCS3 and SOCS6 each had two copies, whereas SOCS5 had three copies. Only single copies of SOCS gene existed in tetrapods (human and mouse), indicating that the multiple copies of SOCS genes in teleost fish could be related to fish-specific whole genome duplication.

3.5. Expression analysis of SOCS genes in healthy fish

The expressions of SOCS genes in healthy tissues of channel catfish were determined. Four SOCS genes were expressed at relatively high levels, with SOCS3a being expressed at the highest level, followed by SOCS3b, CISH, and SOCS1a, among all SOCS genes. The remaining SOCS genes were expressed at relatively low levels (Fig. 4). However, the SOCS genes were overall expressed at relatively low levels in the entire cellular transcriptome level, as the total RPKM value of the 12 SOCS genes was approximately 127.

3.6. Expression analysis of SOCS genes after F. columnare and E. ictaluri infections

Of the 12 catfish SOCS genes, only two genes, *SOCS1a*, and *SOCS3a* were up-regulated after columnaris infection ($p < 0.05$, Table 3). The observed up-regulation occurred early after infection only at 4 h in the gill of catfish after *F. columnare* infection. With the remaining 10 genes, nine were not significantly regulated after *F. columnare* infection, while one, SOCS4, was not included for analysis because its expression reads were lower than the threshold set for the analysis. It is notable that *SOCS1a* and *SOCS3a* were significantly induced

Fig. 1. *Multi-sequence alignment of amino acid sequences of channel catfish SOCS family members.* Conserved SH2 domain and SOCS box are highlighted by solid-lined boxes, as indicated.

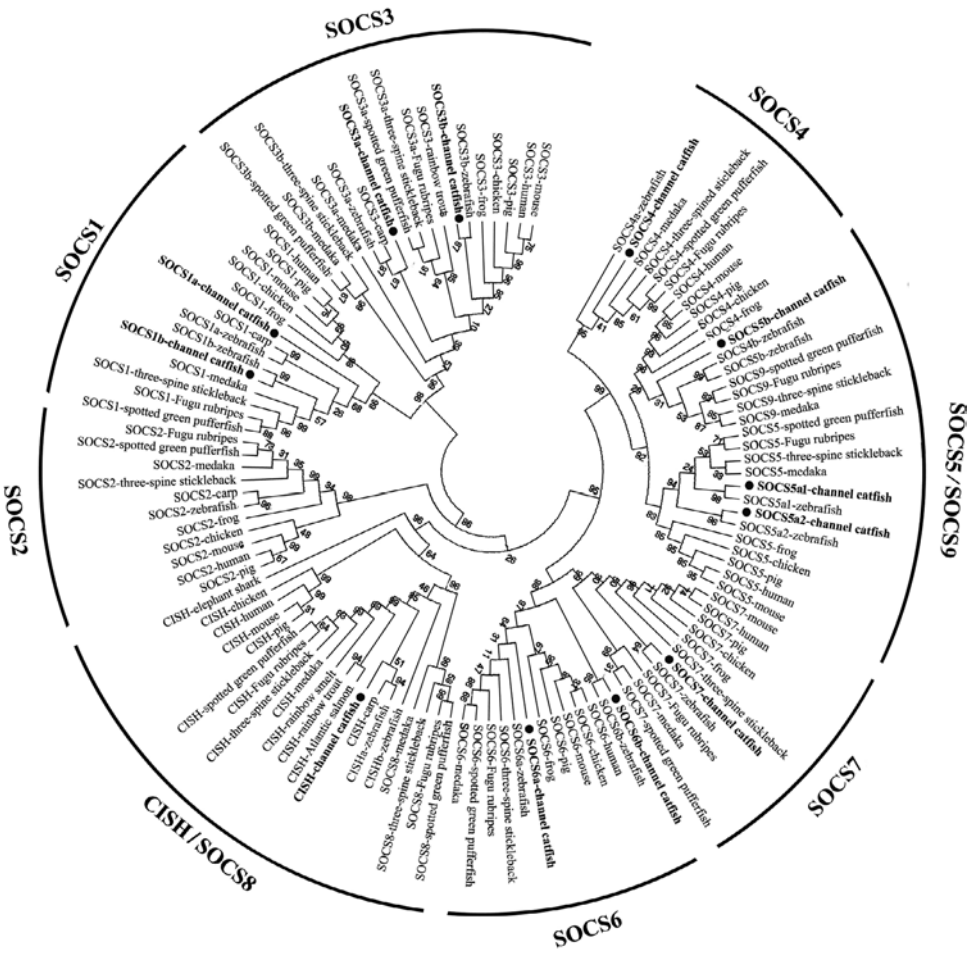

Fig. 2. *Phylogenetic analysis of channel catfish SOCS genes.* The phylogenetic tree was constructed by MEGA 5.0 software using the maximum likelihood method. The bootstrap support for each branch (1000 replications) was shown. The accession numbers of the sequences used for the phylogenetic analysis were provided in supplemental table S1.

at 4 h post-infection, but were down-regulated at later stages of disease progression at 24 h and 48 h post-infection.

Only two SOCS genes, SOCS1a and CISH, were significantly up-regulated in the catfish intestine after *E. ictaluri* infection ($p < 0.05$, Table 4). With both SOCS1a and CISH, their expressions were significantly up-regulated early at 3 h after infection, but were down-regulated later at 24 h and 3 days after infection. Among the remaining 10 SOCS genes, nine were not significantly up-regulated while one was below the minimal reads required for the analysis (Table 4). However, expression of additional four SOCS genes, *SOCS5a1*, *SOCS5a2*, *SOCS6a*, and *SOCS6b*, appeared to be up-regulated although not statistically significant (Table 4).

Table 2
Comparison of SOCS gene copy numbers among various species including human, mouse, chicken, and several fish species. Yellow shading indicated duplicated genes, and gray shading indicates the absence of the specific SOCS genes.

	Human	Mouse	Chicken	Catfish	Zebrafish	Fugu	Medaka	Rainbow trout
SOCS1	1	1	2	2	2	2	2	1
SOCS2	1	1	1	0	1	1	1	1
SOCS3	1	1	1	2	2	1	1	1
SOCS4	1	1	1	1	1	1	1	0
SOCS5/SOCS9	1	1	1	3	3	2	2	1
SOCS6	1	1	1	2	2	1	1	1
SOCS7	1	1	1	1	1	1	1	1
CISH/SOCS8	1	1	1	1	2	2	2	4

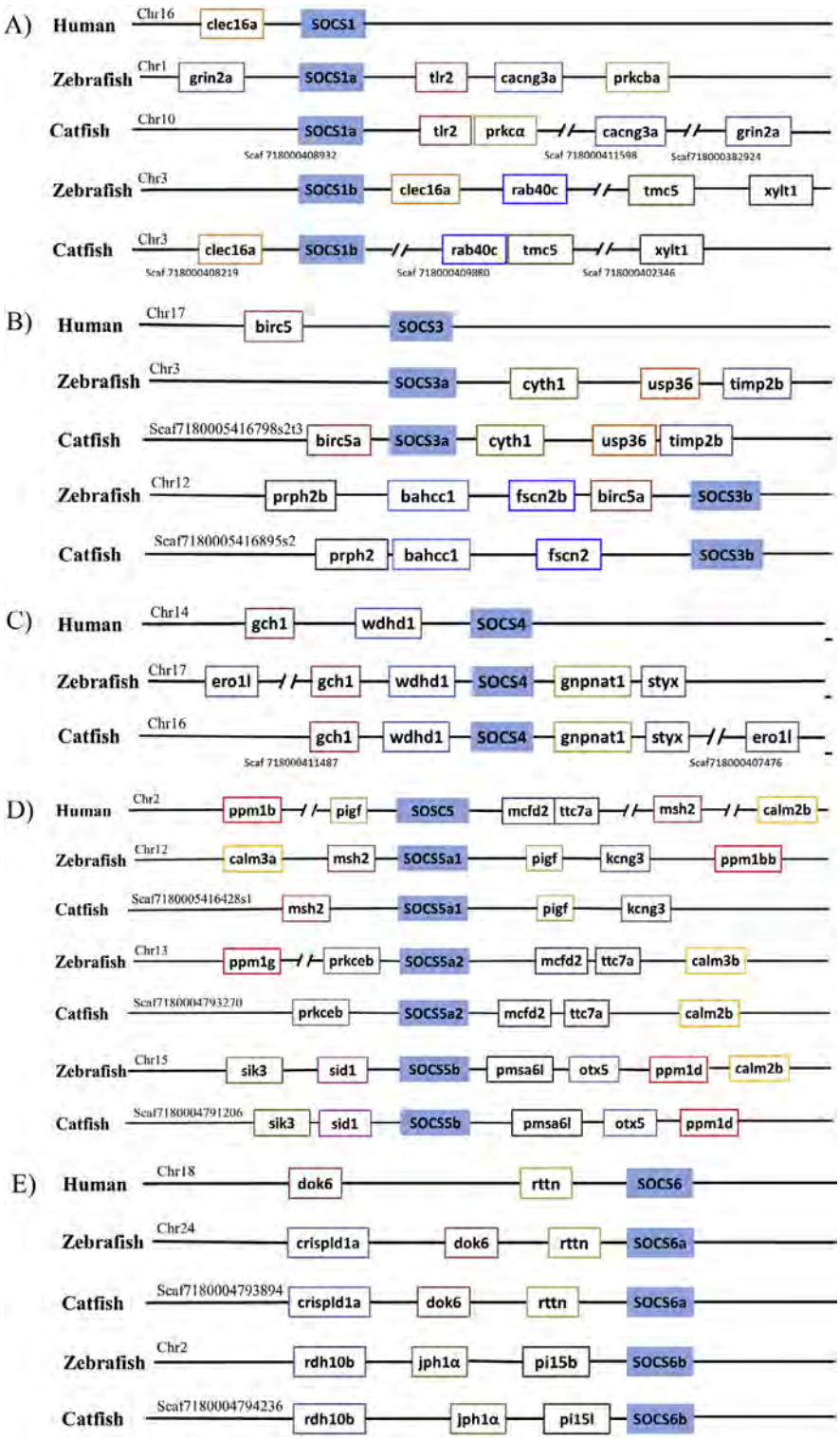

Fig. 3. *Syntenic analyses of SOCS genes using genomic contexts of human, zebrafish, and channel catfish.* (A) SOCS1, (B) SOCS3, (C) SOCS4, (D) SOCS5 and (E) SOCS6. The abbreviations of gene names were celec16a, C-type lectin domain family 16, member a; grin2a, G protein regulated inducer of neurite outgrowth 2; tlr2, toll-like receptor 2; prkcba, protein kinase C, beta a; prkcα, protein kinase C, alpha; cacng3a, calcium channel, voltage-dependent, gamma subunit 3a; rab40c, RAB40C, member RAS oncogene family; tmc5, transmembrane channel-like 5; xylt1, xylosyltransferase 1; bahcc1, BAH domain and coiled-coil containing 1; birc5, baculoviral IAP repeat-containing 5; birc5a, baculoviral IAP repeat-containing 5a; cyth1, cytohesin 1; fscn2, fascin 2; fscn2b, fascin homolog 2; prph2, peripherin 2; prph2b, peripherin 2b; timp2b, tissue inhibitor of metalloproteinase 2b; usp36, ubiquitin specific peptidase 36; gch1, GTP cyclohydrolase 1; ero1l, ERO1-like; wdhd1, WD repeat and HMG-box DNA binding protein 1; gnpnat1, glucosamine-phosphate *N*-acetyltransferase 1; styx, serine/threonine/tyrosine interacting protein; ppm1d, protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1D; calm2b, calmodulin 2b; calm3b, calmodulin 3b; kcng3, potassium voltage-gated channel, subfamily G, member 3; mcfd2, multiple coagulation factor deficiency 2; msh2, mutS homolog 2; otx5, orthodenticle homolog 5; pigf, phosphatidylinositol glycan anchor biosynthesis, class F; pmsa6l, proteasome subunit, α type 6 like; prkceb, protein kinase C, epsilon b; sik3, SIK family kinase 3; sid1, SID1 transmebrane family; ttc7a, tetratricopeptide repeat domain 7A; crispld1α, cysteine-rich secretory protein LCCL domain containing 1α; dok6, docking protein 6; rttn, rotatin; rdh10b, retinol dehydrogenase 10 (all-trans) b; jph1α, junctophilin 1α; pi15, peptidase inhibitor 15.

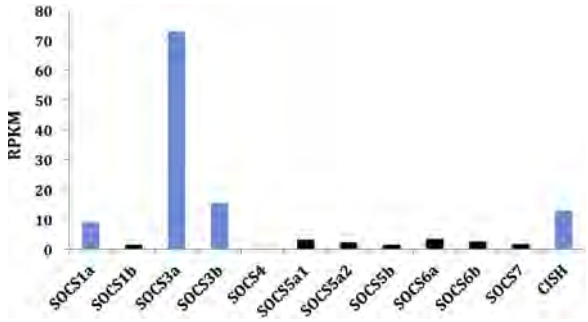

Fig. 4. The expression profiles of SOCS genes in healthy channel catfish. The *y*-axis represents RPKM of SOCS genes and the *x*-axis provided the names of the genes. The blue bars indicated genes expressed at relatively high levels whereas the black bars indicated genes expressed at relatively low levels.

Table 3
Expression of SOCS genes in the gill after *F. columnare* infection at different time points. Asterisks (*) indicated significant differences (p-value < 0.05). Bars indicated that the reads were not high enough to be included in the analysis.

Gene/time	4 h	24 h	48 h
SOCS1a	**2.13***	−1.02	−1.21
SOCS1b	1.71	1.83	−2.11
SOCS3a	**2.24***	1.27	−1.35
SOCS3b	1.43	−1.44	−1.15
SOCS4	–	–	–
SOCS5a1	1.40	1.60	1.34
SOCS5a2	−1.02	−1.15	1.19
SOCS5b	1.14	1.24	1.09
SOCS6a	1.44	1.59	1.45
SOCS6b	1.33	1.34	1.36
SOCS7	1.16	−1.02	−1.12
CISH	−1.01	−1.36	−1.17

4. Discussion

Cytokines are pleiotropic molecules that play critical roles in regulating a vast array of biological processes (Jin et al., 2008). Suppressors of cytokine signaling (SOCS) regulate the responses of immune cells to cytokines (Alexander and Hilton, 2004). In spite of many studies in mammalian species, systematic analysis of these genes among teleost fish species has been lacking. In this study, we identified a total of 12 SOCS genes in the channel catfish genome, conducted phylogenetic and syntenic analyses, and determined the expression profiles of SOCS gene in normal tissues and after bacterial infections. Such genome resource information should be useful for genome analysis and annotation as well as for evolutionary studies in fish species, a group representing over 50% of all vertebrates.

Table 4
Expression of SOCS genes in the intestine after ESC infection. Asterisks (*) indicated significant differences (p-value <0.05). Bars indicated that the reads were not high enough to be included in the analysis.

Gene/time	3 h	24 h	3 Days
SOCS1a	**2.39***	**−2.03***	−1.81
SOCS1b	1.75	−1.10	1.47
SOCS3a	1.56	−1.83	−1.07
SOCS3b	1.27	−1.03	−1.28
SOCS4	–	–	–
SOCS5a1	2.74	2.97	1.36
SOCS5a2	−1.70	2.03	1.67
SOCS5b	1.21	−1.64	1.40
SOCS6a	2.36	3.46	3.00
SOCS6b	1.70	1.71	2.07
SOCS7	−1.84	1.01	−1.44
CISH	**2.16***	−1.66	−1.27

Although exhaustive searches were performed with all catfish genomic resources, the SOCS2 was not found in the channel catfish genome. It is not known at present if SOCS2 was truly missing from the channel catfish genome or it has not been found yet. Among all other SOCS genes, only a single copy existed for each of the SOCS genes in human and mouse, but multiple copies of SOCS genes including SOCS1 (2 copies), SOCS3(2 copies), SOCS5 (3 copies in channel catfish and zebrafish), SOCS6 (2 copies), and CISH (2 copies in many fish species, but only one copy was identified in channel catfish and 4 copies were identified in rainbow trout) (Maehr et al., 2014; Wang et al., 2011). Apparently, several mechanisms may account for the observed differences. These include: (1) the teleost-specific whole genome duplication that may account for the increased copy numbers in general, e.g., 2 copies of many SOCS genes with teleost fish; (2) tetraploidy may account for the high copy numbers within rainbow trout, as the salmonid rainbow trout is generally believed to be tetraploid (Moghadam et al., 2005); (3) lineage-specific gene amplification within this gene family may account for some SOCS genes such as SOCS5/SOCS9, with which three copies existed in the channel catfish and zebrafish genomes; and (4) random genome loss after whole genome duplication in teleosts that may account for the single copy of many SOCS genes within the teleost fish species (Table 2).

The phylogenetic analysis provided insights into the evolution of SOCS genes. The SOCS were clustered into two groups, with the SOCS4, SOCS5, SOCS6, SOCS7 in one group forming the type I family, and the CISH, SOCS1, SOCS2 and SOCS3 forming the type II family (Jin et al., 2008), suggesting that common predecessors of these groups existed (Jin et al., 2008; Zhang et al., 2010). Here, in the phylogenetic analysis, SOCS1 and SOCS3 had a strong supportive sister relationships. A domain structure including a well-defined KIR region in SOCS1 was also identified in the SOCS3, which played important roles in regulating innate and adaptive immune responses (Baker et al., 2009). In addition, the relationship of CISH and SOCS8, as well as SOCS5 and SOCS9 had been discussed in many works. The domain structure, phylogenetic and syntenic analyses of CISH, SOCS8, SOCS5 and SOCS9, indicated that SOCS8 and SOCS9 was a copy of CISH and SOCS5 gene, respectively (Wang et al., 2010, 2011). Fish SOCS9 gene was firstly identified by Jin et al. (2008), and then Wang et al. (2011) reclassified the zebrafish SOCS5a and SOCS5b as SOCS5a1 and SOCS5a2, meanwhile zebrafish SOCS9 was renamed as SOCS5b, based on the structure, phylogenetic and syntenic analyses. However, no strong evidence showed that this nomenclature is suitable in other fish species. Therefore, additional studies are required to provide strong phylogenies of these genes among teleost fishes. In the present work, the syntenic analysis confirmed the annotation of the catfish SOCS5a1, SOCS5a2 and SOCS5b which shared the similar neighboring genes with zebrafish SOCS5a1, SOCS5a2 and SOCS5b, respectively. SOCS5a1, SOCS5a2 and SOCS5b in both catfish and zebrafish shared several neighboring genes with human SOCS5, suggesting the orthologies of tetrapod SOCS5.

To provide insight into functions of SOCS genes, we determined the expression profiles of SOCS genes in healthy channel catfish. The results indicated that SOCS3a was expressed at the highest level in healthy catfish, followed by SOCS1a, SOCS3b and CISH. Studies in healthy trout showed that *SOCS1*, *SOCS2*, *SOCS3*, *SOCS5a2*, *SOCS6* and *SOCS7* were highly expressed in the gill (Wang and Secombes, 2008; Wang et al., 2010). Several investigations suggested that CISH, SOCS1 and SOCS3 were induced by many immune cytokines (Krebs and Hilton, 2001), indicating the functional relationships among these SOCS members. Studies in rat ovary indicated that SOCS1, SOCS3 and CISH were the important regulators of PRLR signal transduction, which expression levels were significant increased during pregnancy (Anderson et al., 2009). Our results were consistent with previous studies where CISH, SOCS1 and SOCS3 were expressed

with similar patterns, which all belong to the type II subfamily (Jin et al., 2008).

We then determined the expression patterns of SOCS genes after *F. columnare* and *E. ictaluri* infections, for the first time in the channel catfish. Channel catfish is the main aquaculture species in the United States (Naylor et al., 2000). The production of channel catfish in the US was severely impacted by columnaris disease. *F. columnare*, an etiological agent of columnaris disease, is pathogenic to many freshwater fish species throughout the world, especially the catfish (Shoemaker et al., 2008). This kind of disease is featured by erosion or necrosis of external tissues, with the gills often being the major site of damage (Farkas and Oláh, 1986). In addition, the intestinal immunology of cellular actors in teleosts was well known for the *E. ictaluri* infection, and the molecular processes underlying bacterial invasion and passage in the intestinal tissues has been studied (Li et al., 2012). In the present study, the expression levels of several SOCS genes were induced after *F. columnare* and *E. ictaluri* infections, suggesting the important roles of these genes in response to bacterial infections. The results were consistent with the observations in the study of Chinese mitten crab after *Listonella anguillarum* infection, in which the induced expression of *SOCS* genes suggested the critical roles in regulating immune defense (Zhang et al., 2010). In this work, the differential expressions of SOCS genes were observed at 4 h after infection with *F. columnare* and *E. ictaluri* infections, suggesting that these SOCS genes may be induced in response to acute bacterial infections. The study in rainbow trout showed that *CISH* was specifically up-regulated by *Yersinia ruckeri* infection (Wang et al., 2010). While the expression of *CISH* in channel catfish was significantly up-regulated at 3 h under *E. ictaluri* infections, which was consistent with the observations in the rainbow trout, but down-regulation of *CISH* was observed throughout the entire *F. columnare* infection challenge, indicating that *CISH* could play distinct roles in response to various bacterial infections. Expressions of SOCS genes examined in *L. anguillarum* bacteria infected fish showed that *SOCS3* was clearly induced in the gill after 24 h (Zhang et al., 2011). In this work, expressions of *SOCS3a* was up-regulated at 4 h after *F. columnare*, then was down-regulated in the following infection treatment. These observations indicated that species-specific expression might exhibit among fish SOCS genes, which could be induced by cytokines, virus, and bacteria (Wang et al., 2011). In addition, the time points when the expression was analyzed may have led to the various observed expression levels. In channel catfish, significantly regulated genes, with both columnaris and ESC involving SOCS1a, SOCS3a, and CISH genes, tended to be up-regulated early after infection, but down-regulated at later stages of pathogenesis. This pattern of regulation may well be correlated with innate immune responses involving massive expression of many chemokines and cytokines after bacterial infection (Bao et al., 2006; Peatman et al., 2006; Peatman and Liu, 2007). In addition to the three significantly regulated genes, four additional SOCS genes, *SOCS5a1*, *SOCS5a2*, *SOCS6a*, and *SOCS6b* were observed to be up-regulated, but were not statistically significant. These genes may indeed be up-regulated, but their statistical significance was influenced by their relative low expression. With meta-analysis using RNA-Seq, the power to detect statistical significance is related to the expression level. For instance, if a RPKM of a gene is changed by two-fold from 2 to 4, they may not be statistically significant even though the absolute fold change is two. In contrast, if the RPKM is increased from 20,000 to 40,000, even though the absolute fold change is also two, it is highly likely that in this case the expression difference would be statistically significant. Assuming these genes were genuinely up-regulated, our observations were consistent with the report from previous studies, in which the mRNA expression of SOCS5 and SOCS6 showed similar patterns in most tested tissues, suggesting their transcriptional co-regulation (Yoon et al., 2012).

In conclusion, suppressors of cytokine signaling negatively regulate cytokine signaling, and are involved in controlling excessive cytokine effects (Jin et al., 2008). In this work, 12 members of SOCS genes in channel catfish were identified, annotated and characterized, and the expression profiles were determined after infections with *F. columnare* and *E. ictaluri*. This work set the foundation for further studies to understand mechanisms of SOCS involved in the regulation of immune responses in fish species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molimm.2014.12.003.

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