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RESEARCH ARTICLE

Molecular characterization of two suppressor of cytokine signaling 1 genes (*SOCS1a* and *SOCS1b*) in chickens

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Abstract Suppressor of cytokine signaling 1 (SOCS1) protein can inhibit the signal transduction triggered by some cytokines or hormones and thus are important in many physiological/pathological processes, including innate and adaptive immunity, inflammation, and development in mammals. However, there is sparse information about their structure, tissue expression, in birds, where their biological functions remain unknown. In this study, we cloned and characterized two *SOCS1* genes (named *cSOCS1a* and *cSOCS1b*) from chickens. *SOCS1a* is predicted to encode a 207-amino acid protein, which shares high amino acid sequence identity (64%–67%) with human and mouse SOCS1. Besides *SOCS1a*, a novel *SOCS1b* gene was also identified in chickens and other non-mammalian vertebrates including *Xenopus tropicalis*. Chicken *SOCS1b* is predicted to encode a 212-amino acid protein, which shares only 30%–32% amino acid sequence identity with human SOCS1 and cSOCS1a. RT-PCR assay revealed that both *cSOCS1a* and *cSOCS1b* are widely expressed in all chicken tissues. Using a luciferase reporter assay system, we further demonstrated that transient expression of *cSOCS1a* and *cSOCS1b* can significantly inhibit chicken growth hormone (GH)- or prolactin (PRL)-induced luciferase activities of Hep G2 cells expressing cGH receptor (or cPRL receptor), indicating that SOCS1a and SOCS1b proteins can negatively regulate GH/PRL signaling. Taken together, these data suggest that both cSOCS1a and cSOCS1b may function as negative regulators of cytokine/hormone actions, such as modulation of GH/PRL actions in chickens.

Keywords chicken, SOCS1a, SOCS1b, growth hormone, prolactin

1 Introduction

Suppressor of cytokine signaling (SOCS) proteins are a family of intracellular molecules that can inhibit the signal transduction of many cytokines, growth factors, and hormones[1]. There are 8 SOCS proteins, SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, SOCS7, and cytokine-induced SRC-homology 2 (SH2) protein (CIS), that have been identified in mammals[1,2]. SOCS1 was discovered independently by three laboratories in 1997 and proved to be responsible for the negative regulation of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway triggered by cytokines, growth factors and hormones[1,3–5]. Like other SOCS proteins, SOCS1 possesses three domains: an N-terminal domain including the kinase inhibitory region (KIR)[6], a central Src homology 2 (SH2) domain, and a C-terminal 40-amino acid module known as the SOCS box region[7,8]. The central SH2 domain and N-terminal region have been shown to be important for binding to JAKs and inhibiting the signal transduction[6], while the SOCS box can interact with elongin B, elongin C, cullin 5 and RING box 2 (RBX2), and thus mediate proteasomal degradation of the associated signaling complex[9,10].

As one of the most important negative regulators of cytokine signaling, SOCS1 is reported to be important in many physiological/pathological processes in mammals, such as innate and adaptive immunity, inflammation, hepatitis-induced carcinogenesis, myeloid leukemia, and metabolism syndromes[2,11]. Mice lacking the *SOCS1* gene (*SOCS1*$^{-/-}$) die at three weeks of age and display severe lymphopenia and macrophage infiltration of major organs[12–14], further emphasizing its importance in immunity[11]. Interestingly, SOCS1 has also been reported to inhibit the JAK-STAT signaling pathway activated by growth hormone and prolactin[15,16], the two peptide hormones mainly produced by the vertebrate anterior

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pituitary[17], implying a potential role of SOCS1 in modulating their actions, such as controlling growth and reproduction. However, this possibility has received little attention.

In contrast to the extensive study of SOCS1 in mammals, little is known about the structure, expression, and physiological roles of *SOCS1* in non-mammalian vertebrates. Recently, a *SOCS1* gene has been identified in several teleost species[18,19]. *In vitro* studies also suggest a possible role of *SOCS1* in fish immunity[20,21]. However, the limited information on *SOCS1* from non-mammalian vertebrates greatly limits our understanding of the conserved physiological roles of *SOCS1* across vertebrates. Therefore, using chicken as an experimental model, the present study aimed to: (1) clone *SOCS1* gene from chicken and examine its tissue expression; (2) examine its potential roles in GH/PRL signaling *in vitro*. The results revealed for the first time that two *SOCS1* genes (*SOCS1a* and *SOCS1b*) exist in chickens and other non-mammalian vertebrates including frogs. Functional studies showed that they can inhibit chicken GH/PRL signaling and thus may have physiological roles in chickens, such as controlling growth and reproduction.

2 Materials and methods

2.1 Chemicals, hormones, and primers

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO), and restriction enzymes were obtained from Amersham Biosciences (GE Healthcare Bio-Sciences Corp, Piscataway, NJ) unless stated otherwise. Recombinant chicken prolactin (cPRL) and growth hormone (cGH) were prepared as previously reported[22]. All primers were synthesized by Invitrogen and are listed in Table 1.

2.2 Total RNA extraction

Adult chickens were killed, and different tissues, including brain, heart, small intestine, kidney, liver, lung, muscle, ovary, testis, pituitary and spleen, were collected and stored at −80°C until used. Total RNA was extracted from chicken tissues with RNAzol Reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer's instructions and resuspended in H_2O treated with diethyl pyrocarbonate. All animal experiments

Table 1 Primers used in this study[a]

Primers	Sense/Antisense	Primer sequence (5′ to 3′)	Size/bp
Primers for rapid amplification of 5′-ends (5′-RACE)			
cSOCS1a-L1	Antisense	TCCAGCAGGCTGCTTGCTCGAGTGA	
cSOCS1a-L2	Antisense	CTTCTGCGTGCTGTCCCTGATGAGGA	
cSOCS1b-L1	Antisense	AGCTGTCCCGCACCAGGTAGGTGCCA	
cSOCS1b-L1	Antisense	AGTCGGCATCCGCACGCTCAGGCTGA	
Primers for constructing the expression plasmids[b]			
cSOCS1a	Sense	CGGGGTACCGCTGGCCTAGGCTGTAGGAT	701
	Antisense	CGGGAATTCACACATCTCTCACATGTCTCT	
cSOCS1b	Sense	CGGGGTACCGGGATCCATGGGCTCTTTGA	724
	Antisense	CGGGAATTCTCCAGCATGGCTGTGTGCAT	
cGHR	Sense	CGGGGTACCTGCTGACATTTGAGAAT	1868
	Antisense	CCGCTCGAGAATTGCTACGGCATGAT	
cPRLR	Sense	CGGGGTACCAAGAGGAAGTGGAAATCATGA	2556
	Antisense	CCGGAATTCTGTAGCATTACCTGATGAAGAG	
Primers for RT-PCR assays			
cSOCS1a	Sense	GCTGGCCTAGGCTGTAGGAT	688
	Antisense	ACACATCTCTCACATGTCTCT	
cSOCS1b	Sense	GGGATCCATGGGCTCTTTGA	710
	Antisense	TCCAGCATGGCTGTGTGCAT	
β-actin	Sense	TGTGCTACGTCGCACTGGAT	401
	Antisense	GCTGATCCACATCTGCTGGA	

Note: [a]All primers were synthesized by Invitrogen (China); [b]restriction sites added to the 5′-end of the primers are underlined.

were performed according to the guidelines provided by the Animal Ethics Committee of Sichuan University.

2.3 Reverse transcription and polymerase chain reaction assay

Reverse transcription (RT) was performed at 42°C for 2 h in a total volume of 10 μL consisting of 2 μg total RNA from different tissues, 1 × Single Strand Buffer, 0.5 mmol·L^{-1} each deoxynucleotide triphosphate, 0.5 μg oligo-deoxythymide, and 100 U Moloney murine leukemia virus reverse transcriptase (Promega, Madison, MI). All negative controls were performed under the same conditions, but without the addition of reverse transcriptase.

Reverse transcription-PCR assays were performed to examine mRNA expression of *cSOCS1a* and *cSOCS1b* in chicken tissues according to the previously established method[22]. For *cSOCS1a* gene, 33 cycles of 30 s at 95°C, 60 s at 60°C, and 60 s at 72°C were used followed by a 5 min extension at 72°C. For *cSOCS1b* gene, 33 cycles of 30 s at 95°C, 60 s at 61°C, 60 s at 72°C were used followed by a 5 min extension at 72°C. For *β-actin* gene (used as an internal control), 23 cycles of 30 s at 95°C, 30 s at 58°C, 60 s at 72°C were used followed by a 5 min extension at 72°C. The primers used are listed in Table 1. The PCR products were visualized on a UV-transilluminator (Bio-Rad Laboratories, Inc. Hercules, CA) after electrophoresis on 2% agarose gel containing ethidium bromide. To confirm the specificity of the PCR reaction, the identity of PCR products was verified by sequencing.

2.4 Cloning the cDNAs of *cSOCS1a* and *cSOCS1b*

According to the predicted cDNA sequence of chicken *SOCS1* (called *SOCS1a* here) (GenBank accession no.: XM_414929) deposited in GenBank, or the genomic sequence of the novel *SOCS1*-like gene (called *SOCS1b* here) located on chicken chromosome 1 (http://www.ensembl.org/Gallus_gallus), gene-specific primers were designed to amplify the cDNAs covering an open reading frame of *cSOCS1a* or *cSOCS1b* from adult chicken liver with the use of high-fidelity Taq DNA polymerase (TOYOBO) (Table 1). The amplified PCR products were cloned into pTA2 vector and sequenced by ABI3100 Genetic Analyzer (BGI, Shanghai, China).

2.5 Rapid amplification of 5′-cDNA ends (5′-RACE) of chicken *SOCS1a* and *SOCS1b*

To determine the 5′-untranslated region (5′-UTR) of chicken *SOCS1a* and *SOCS1b* genes, gene-specific primers were designed to amplify the 5′-UTRs of c*SOCS1a* and c*SOCS1b* from adult chicken liver by using SMART-RACE cDNA amplification Kit (Clontech, Palo Alto, CA). The amplified PCR products were cloned into pTA2 vector (TOYOBO, Japan) and sequenced by ABI3100 Genetic Analyzer (BGI).

2.6 Data mining, sequence alignment, and phylogenetic analysis

To determine whether *SOCS1a* or *SOCS1b* genes also exist in other vertebrate species, using the cDNA sequences from chickens as references, we performed a blast search in the publicly available genomes (http://www.ensembl.org) and identified *SOCS1b* and/or *SOCS1a* genes in non-mammalian vertebrate species, including *Xenopus tropicalis* and coelacanth. The amino acid sequences of *SOCS1* genes were aligned using the ClustalW program (BioEdit, Carlsbad, CA). Phylogenetic analysis was computed using the program MEGA5, in which the phylogenetic tree was constructed using the Neighboring-Joining (NJ) method with 1000 bootstrap replicates.

2.7 Evaluation of the inhibitory effects of cSOCS1a and cSOCS1b on cGH/cPRL signaling in cultured Hep G2 cells

According to the cDNA sequences of chicken *SOCS1a*, *SOCS1b*, growth hormone receptor (cGHR), and prolactin receptor (cPRLR), gene-specific primers were designed to amplify the ORF of each gene from chicken liver or kidneys using high-fidelity Taq DNA polymerase (TOYOBO) (Table 1). The amplified PCR products were cloned into the pcDNA3.1 (+) expression vector (Invitrogen) and sequenced. These expression plasmids were then used in the following experiments.

To test whether cSOCS1a and cSOCS1b proteins are capable of inhibiting cGH/cPRL signaling *in vitro*, *cSOCS1a* or *cSOCS1b* were transiently expressed in human hepatocellular carcinoma (Hep G2) cells expressing either cGHR or cPRLR, and the inhibitory action on cGHR- or cPRLR-mediated signaling evaluated by a 5 × STAT5–luciferase reporter system established in previous studies, which have been shown to be capable of monitoring the receptor-activated JAK-STAT signaling pathway[22–24]. In brief, Hep G2 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 U·mL^{-1} of penicillin G, and 100 mg·mL^{-1} of streptomycin (HyClone, Logan, UT, USA) in 48-well plate (Nunc, Rochester, NY, USA) incubated at 37°C with 5% CO_2. At 70% confluency, Hep G2 cells were co-transfected with 100 ng of 5 × STAT5–luciferase reporter construct (an artificial promoter construct containing five STAT5-response elements fused to the luciferase gene), 33 ng (or 167 ng) of *cSOCS1a* (or *cSOCS1b*) expression plasmid, 20 ng of expression plasmid encoding cPRLR (or cGHR), 10 ng of expression plasmid encoding pig STAT5a, and 10 ng of pRL-TK vector using Lipofectamine (Invitrogen). After 24 h of culture, cells were then treated with recombinant chicken growth hormone

(cGH, 200 ng· mL^{-1}) or chicken prolactin hormone (cPRL, 200 ng·mL^{-1}) (or hormone-free medium used as control) for 18 h at 37°C before being harvested for luciferase assay. After removal of culture medium, Hep G2 cells were lysed by adding 100 μL of 1 × Passive Lysis Buffer per well, and the luciferase activity of 15 μL of cellular lysates was determined using Dual Luciferase Assay Kit according to the manufacturer's instructions (Promega).

2.8 Data analysis

Luciferase activity of Hep G2 cells in each treatment group was normalized to *Renilla* luciferase activity derived from the pRL-TK vector and then expressed as the relative increase compared with the control group (without treatment). The data were analyzed by one-way ANOVA followed by the Newman-Keuls test to compare all pairs of groups using GraphPad Prism 5 (GraphPad Software, San Diego, CA). To validate the results, all experiments were repeated two or three times.

3 Results

3.1 Cloning of the *SOCS1a* and *SOCS1b* genes in chickens

According to the predicted cDNA sequence of chicken *SOCS1* gene deposited in GenBank (XM_414929), using RT-PCR, we amplified and cloned the cDNA sequence (688 bp) containing an ORF of *SOCS1* from chicken liver. The cloned SOCS1 is 207 amino acids long and shares a high degree of amino acid sequence identity with that of humans (64%, NM_003745), mice (67%, NM_009896) and pigs (63%, HM462248) (Fig. 1a). Like mammalian SOCS1, chicken SOCS1 includes a central SH2 domain, a C-terminal SOCS box, and an N-terminal domain, within which the conserved KIR and extended SH2 subdomain (ESS) have also been identified[2,6].

In addition to the *SOCS1* gene, a novel *SOCS1*-like gene was also found on chicken chromosome 1. Therefore, this novel *SOCS1*-like gene is designated as the chicken *SOCS1b* (*cSOCS1b*) in this study, whereas the above-mentioned chicken *SOCS1* orthologous to human *SOCS1* is defined as the *SOCS1a* gene (*cSOCS1a*). Using RT-PCR, we cloned the *SOCS1b* cDNA from chicken liver, which is 710 bp in length (accession no.: HQ917699) and predicted to encode a 212-amino acid protein. Although cSOCS1b shares only 30%–32% amino acid identity with human SOCS1 and cSOCS1a, like cSOCS1a, cSOCS1b also contains a KIR, an ESS subdomain, a central SH2 domain, and a C-terminal SOCS box (Fig. 1b).

3.2 Characterization of 5′-untranslated region of *cSOCS1a* and *cSOCS1b*

Like mammalian *SOCS1*, the coding regions of *cSOCS1a* and *cSOCS1b* genes are intronless. To examine whether additional exon(s) are located upstream of the translation start site (ATG) of the two *SOCS1* genes, 5′-RACE PCR was performed to clone the 5′-UTR of each *SOCS1* gene from chicken liver. Comparison of the cloned 5′-UTRs with the chicken genome revealed that *cSOCS1a* 5′-UTR is 100 bp long and contains a non-coding exon (exon 1, 25 bp) upstream of the translation start site, whereas the *cSOCS1b* gene has a 5′-UTR of 254 bp and three exons, including two non-coding exons (exon 1, 67 bp; exon 2, 121 bp) upstream of the ATG codon. The exon organization of *cSOCS1a* and *cSOCS1b* is schematically depicted in Fig. 2.

3.3 Discovery of the novel *SOCS1b* gene in other non-mammalian vertebrate species

To examine whether the novel *SOCS1b* gene exists in other vertebrate species, using chicken *SOCS1b* as a reference, we searched the genome database of several vertebrate species including humans, American alligators, *Xenopus tropicalis*, coelacanth, zebrafish and several avian species. In addition to the identification of *SOCS1a* gene orthologous to human *SOCS1* in these species (Fig. 1a, Fig. 3a), a novel *SOCS1b* highly homologous to *cSOCS1b* (45%–87% amino acid identity) could also be identified in *Xenopus tropicalis*, American alligators, coelacanths and all avian species examined (Fig. 1b, Fig. 3b). In contrast, the *SOCS1b* gene seemed to be lost in humans and zebrafish, as suggested by synteny analysis (Fig. 3b).

3.4 Tissue distribution of *cSOCS1a* and *cSOCS1b* mRNA

To elucidate the potential role of the two *SOCS1* genes in chickens, using RT-PCR, the mRNA expression of *cSOCS1a* and *cSOCS1b* was examined in brain, heart, intestines, kidney, liver, lung, muscle, ovary, testis, pituitary and spleen tissue from adult chickens. As shown in Fig. 2**,** the mRNA expression of both *cSOCS1a* and *cSOCS1b* is widely expressed in all tissues examined.

3.5 Functional characterization of *cSOCS1a* and *cSOCS1b* in cultured Hep G2 cells

It has been reported that SOCS1 can negatively regulate GH or PRL signaling in mammals[15,16], therefore, in this study, we also examined whether transient expression of *cSOCS1a* or *cSOCS1b* in Hep G2 cells can block cGH/cPRL signaling using a 5 × luciferase reporter system, which can sensitively monitor the receptor (cGHR or cPRLR)-activated JAK-STAT signaling pathway, as reported in previous studies[22,24].

As showed in Fig. 4, recombinant chicken GH (or cPRL) treatment (200 ng·mL^{-1}, 18 h) can significantly stimulate luciferase activities of HepG2 cells expressing cGHR (or cPRLR), indicating that cGHR (or cPRLR) activation by

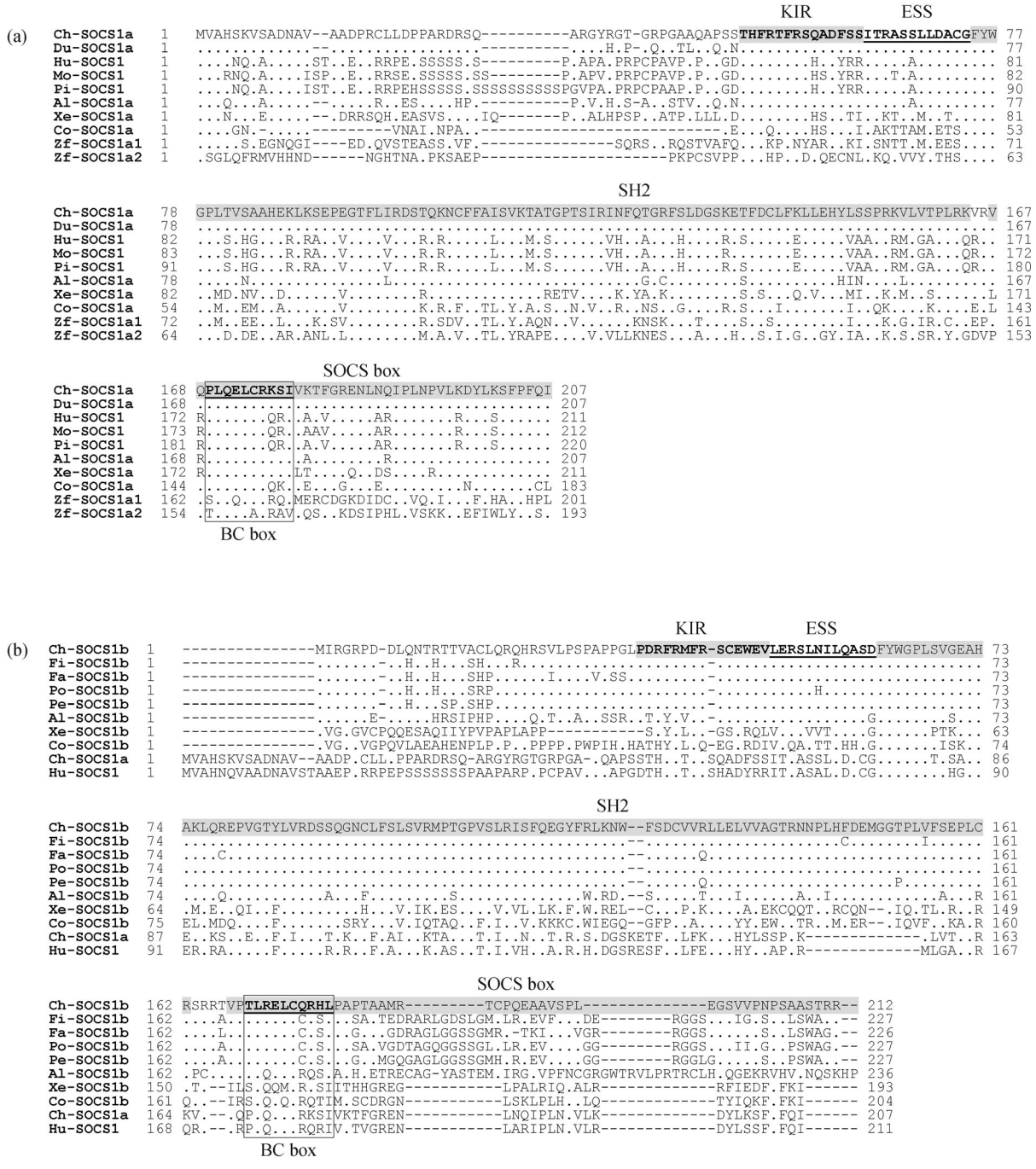

Fig. 1 Amino acid sequence alignment of SOCS1 proteins from chicken and other species. (a) Alignment of chicken SOCS1a (Ch-SOCS1a, XM_414929) with SOCS1a (or SOCS1) from duck (Du-SOCS1a: XP_005027549), human (Hu-SOCS1), mouse (Mo-SOCS1), pig (Pi-SOCS1), alligator (Al-SOCS1a), *Xenopus tropicalis* (Xe-SOCS1a), coelacanth (Co-SOCS1a) and zebrafish (zf-SOCS1a1, NM_001003467; zf-SOCS1a2, JN800507); (b) alignment of chicken SOCS1b (Ch-SOCS1b, HQ917699) with that of zebra finch (Fi-SOCS1b), falcon (Fa-SOCS1b), pigeon (Po-SOCS1b), penguin (Pe-SOCS1b), alligator (Al-SOCS1b), *Xenopus tropicalis* (Xe-SOCS1b), coelacanth (Co-SOCS1b), or with that of chicken SOCS1a (Ch-SOCS1a) or human SOCS1 (Hu-SOCS1). Dots indicate the amino acid residues identical to chicken SOCS1a (or SOCS1b) and dashes represent gaps in the alignment. The conversed SH2 domain, and SOCS box are shaded; the kinase inhibitory domain (KIR) is shaded and shown in bold; the Extended SH2 domain (ESS) is bold and underlined; the BC box is shaded and boxed. The amino acid sequences of *SOCS1a* or *SOCS1b* genes from other species were either retrieved from GenBank or predicted according to genomic sequences.

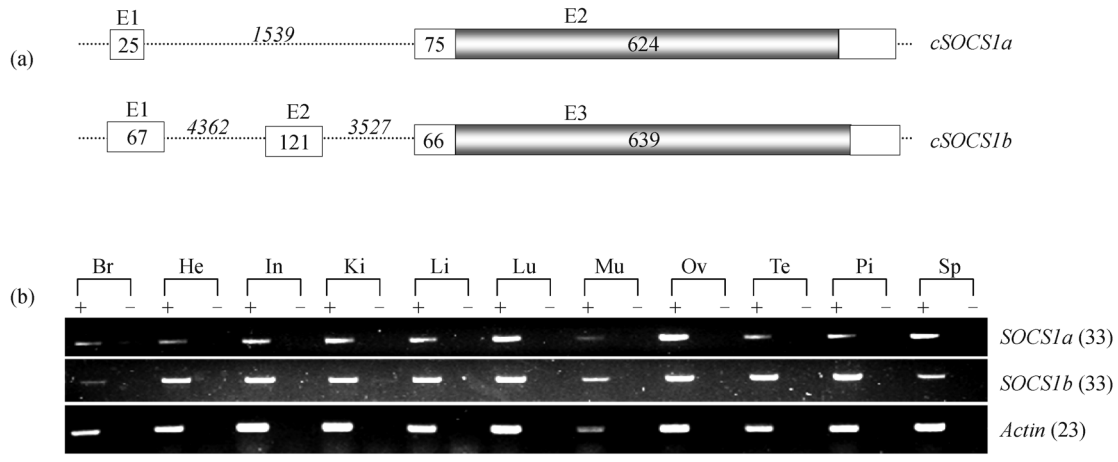

Fig. 2 Gene structure (a) and tissue expression (b) of chicken *SOCS1a* and *SOCS1b*. (a) Exon (E) organization of *cSOCS1a* and *cSOCS1b* genes. The coding regions of *cSOCS1a* and *cSOCS1b* are intronless and shaded. *cSOCS1a* contains one non-coding exon (Exon 1, 25 bp) upstream of the translation start site (ATG), while cSOCS1b contains two non-coding exons (Exon 1, 67 bp; Exon 2, 121 bp) upstream of ATG codon. The numbers in the boxes indicate the size of non-coding or coding regions (shaded), and the number in italics indicates the size of the intron; (b) RT-PCR detection of *cSOCS1a* and *cSOCS1b* mRNA expression in adult chicken tissues, including the brain (Br), heart (He), small intestine (In), kidneys (Ki), liver (Li), lung (Lu), muscle (Mu), ovary (Ov), testes (Te), pituitary (Pi), and spleen (Sp). No PCR band was detected in the negative controls (–). Numbers in parenthesis indicate the PCR cycles used.

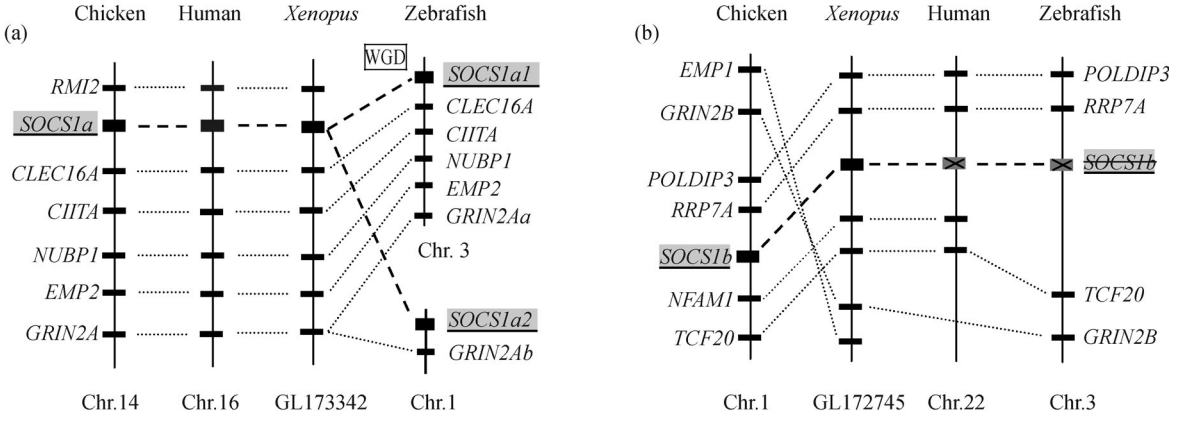

Fig. 3 Synteny analyses showing the existence of *SOCS1a* (a) and *SOCS1b* (b) in non-mammalian vertebrates. (a) *SOCS1a* gene is located in a syntenic region conserved between chickens, humans, *Xenopus tropicalis* and zebrafish. The two copies of *SOCS1a* (*SOCS1a1*, NM_001003467; *SOCS1a2*, JN800507) genes in zebrafish are likely to be paralogs generated by the teleost-specific whole genome duplication (WGD) event; (b) *SOCS1b* gene is located in a syntenic region conserved between chickens and *Xenopus tropicalis*. *SOCS1b* gene is likely lost in zebrafish and human genomes. Dashed lines denote the genes of interest (*SOCS1a* or *SOCS1b*), while dotted lines indicate the syntenic genes identified in these species. Genes were labeled according to their gene symbols in the human genome. Gray bars appeared in (b) indicate the *SOCS1b* gene likely lost in these species. *SOCS1a* gene identified in chickens, *Xenopus tropicalis*, and zebrafish is orthogous to mammalian (human) *SOCS1* gene. Chr, chromosome; WGD, teleost-specific whole genome duplication.

their specific ligands can activate the JAK-STAT signaling pathway, as previously reported[22,24]. However, transient expression of cSOCS1a completely inhibited the stimulatory effect of cGH- or cPRL-induced luciferase activities of Hep G2 cells. Likewise, cSOCS1b was also shown to inhibit GH- or PRL-induced luciferase activities of HepG2 cells, indicating that like SOCS1a, SOCS1b can also negatively regulate cGH/cPRL signaling. Moreover, we also noted that cSOCS1a protein appeared to be much more effective than cSOCS1b in blocking cGH/cPRL signaling, since transfection of 33 ng cSOCS1a plasmid into Hep G2 cells can completely abolish hormone action, while the transfection of the same amount of cSOCS1b expression plasmid only partially attenuated GH/PRL signaling (Fig. 4). The transient expression of cSOCS1a or SOCS1b, as a control, in the absence of hormone, only slightly inhibited the basal luciferase activity of Hep G2 cells expressing cGHR (or cPRLR) (data not shown).

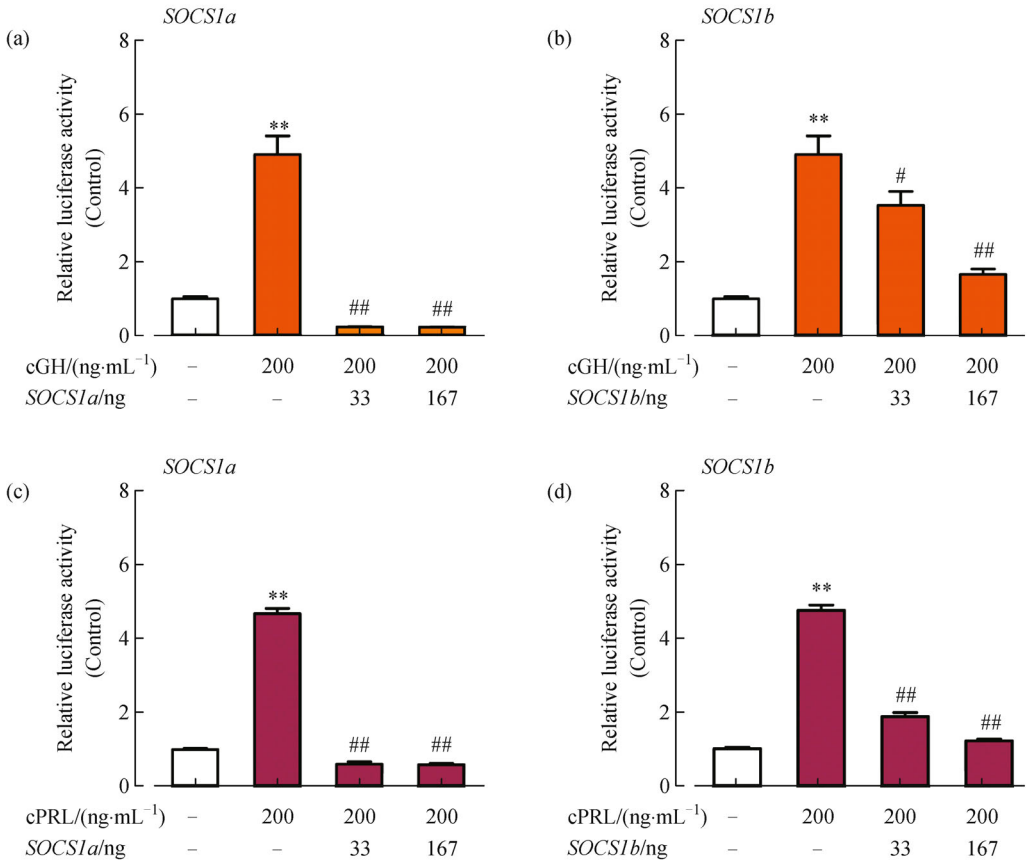

Fig. 4 Inhibition of GH/PRL signaling by *cSOCS1a* (a, c) and *cSOCS1b* (b, d). (a, b) transient expression of *cSOCS1a* or *cSOCS1b* inhibits cGH-induced (200 ng·mL^{-1}, 18 h) luciferase activities of Hep G2 cells expressing chicken GH receptor, monitored by a 5 × STAT luciferase reporter system; (c, d) transient expression of *cSOCS1a* or *cSOCS1b* inhibits cPRL-induced (200 ng·mL^{-1}, 18 h) luciferase activities of Hep G2 cells expressing chicken PRL receptor, monitored by a 5 × STAT luciferase reporter system. The luciferase activity of Hep G2 cells in each treatment group was expressed as relative increase compared to the control group (without hormone treatment). Each data point represents mean±SEM of three replicates. **, $P < 0.001$ vs control (without hormone treatment); #, $P < 0.01$; ##, $P < 0.001$ vs hormone treatment group (without *SOCS1a*/*SOCS1b* added).

4 Discussion

Two *SOCS1* genes, *SOCS1*a and *SOCS1b*, were identified in chickens. RT-PCR assay revealed that these two genes were widely expressed in all chicken tissues examined. Functional studies showed that both SOCS1s are active and capable of attenuating cGH/cPRL signaling in cultured Hep G2 cells. Notably, the novel *SOCS1b* could also be identified in other non-mammalian vertebrates including frogs. To our knowledge, this study is the first to report that two functional *SOCS1* genes co-exist in the non-mammalian vertebrates including chickens, and that they may negatively regulate GH/PRL signaling.

4.1 Identification of *SOCS1a* and *SOCS1b* in chickens and other non-mammalian vertebrates

Since *SOCS1* was identified in 1997[3–5], there has been growing evidence that SOCS1 in mammals can suppress the signaling of many cytokines (including interleukins and interferons), and thus regulate innate and adaptive immunity[1,11]. Moreover, SOCS1 has also been suggested to inhibit the signaling transduction of growth hormone[15], and prolactin[16]. To our knowledge, however, studies on the structure and biological functions of SOCS1 in non-mammalian vertebrates including birds is rare, notwithstanding several studies showing that *SOCS1* gene may have a conserved role in fish immunity, as in mammals[20,21]. In this study, we identified *SOCS1a* and *SOCS1b* in chickens. Chicken *SOCS1a* shares high amino acid sequence identity with its mammalian counterpart (64%–67%), and a remarkable degree of conservation was found in the SH2, ESS and KIR domains between chicken SOCS1a and mammalian SOCS1. This indicates that, as in mammals[6,25,26], these cSOCS1a domains are likely involved in binding to JAK kinase, thus blocking JAK-mediated signaling in chickens. This speculation is supported by the fact that the transient expression of

cSOCS1a can effectively inhibit GH/PRL signaling (Fig. 4). Furthermore, a conserved BC box motif, known to be critical for mediating elongin B/C binding, has been found in chicken SOCS1a (Fig. 1)[27], also hinting that the BC box motif of cSOCS1a may have a role in mediating the proteasomal degradation of the associated signaling molecules, as reported in mammals[10].

Besides *cSOCS1a*, a novel *SOCS1*-like gene, named *SOCS1b*, was also identified in the present study. Although SOCS1b shares only 30%–32% identity with human SOCS1 and chicken SOCS1a, it also contains SH2, ESS, and KIR domains, which show relatively high amino acid sequence identity with those of chicken SOCS1a (Fig. 1b). Moreover, a conserved BC box motif was noted in the C-terminal region of cSOCS1b. The existence of these conserved structural motifs (Fig. 1b), together with the evidence showing that cSOCS1b can attenuate GH/PRL signaling in Hep G2 cells, also suggests that like cSOCS1a, cSOCS1b is likely to be a functional protein *in vivo*.

As in chickens, the novel *SOCS1b* gene could also be identified in other non-mammalian species including frogs and coelacanths. This finding, together with the conservation of structural motifs noted between SOCS1a and SOCS1b, led us to hypothesize that the two *SOCS1* genes identified in chickens and other non-mammalian vertebrates were likely to have originated by a gene duplication event in the last common ancestor of tetrapods and teleosts, probably by a whole genome duplication, or a chromosomal duplication event in the course of vertebrate evolution[28]. This hypothesis is supported by the identification of several paralogous genes (e.g., *EMP1* and *EMP2*, *GRIN2A* and *GRIN2B* genes) adjacent to both *SOCS1* genes in chicken and *Xenopus* genomes, as well as the closer evolutionary relationship of *SOCS1b* to *SOCS1a*, than to other SOCS family members (including SOCS2, SOCS3 and CIS) as revealed by our phylogenetic analysis (Fig. 5). *SOCS1b* has not been identified in mammalian genomes, although its neighboring genes, such as *RRP7A* and *TCF20* genes can be identified in humans (Fig. 3), suggesting that *SOCS1b* has been lost in mammalian lineages during evolution.

Two *SOCS1* genes were also identified in zebrafish chromosomes 1 and 3, respectively in this study (NM_001003467; JN800507); however, they were most likely generated by the fish-specific genome duplication event[29], as suggested by the synteny analysis (Fig. 3). Therefore, the two zebrafish *SOCS1* genes are called *SOCS1a1* and *SOCS1a2* in this study (Fig. 1). In contrast, the *SOCS1b* gene may have been eliminated from the zebrafish genome during evolution.

4.2 Tissue expression of the two *cSOCS1* mRNAs: implication for their potential inhibitory action on GH/PRL signaling

In this study, both *SOCS1* genes were found to be widely expressed in all chicken tissues examined. This finding is consistent with the observation in humans, mice, and teleosts[1,18,20,21]. The ubiquitous expression of both *cSOCS1* genes implies that as in mammals, *cSOCS1s* may be important in a variety of tissues by blocking the JAK-STAT signaling pathway triggered by cytokines, growth factors, and peptide hormones[1]. Although it remains unclear whether cytokines or hormones can induce *SOCS1* expression in chicken tissues, as demonstrated in mammals and fish[1,20,21], within the *cSOCS1a* promoter region, a canonical STAT-response element near exon 1 was identified. This also hints that *cSOCS1a* expression may be induced by cytokines/hormone through activation of the JAK-STAT signaling pathway, thus leading to feedback inhibition of cytokine/hormone signaling.

In mammals and teleosts, SOCS1 is a critical inhibitor in cytokine signaling[11]. However, its action on GH/PRL signaling in vertebrates is not fully understood. In this study, the transient expression of cSOCS1a completely blocked both cPRLR- and cGHR-mediated signaling in Hep G2 cells. This finding is consistent with two studies in mammals, in which SOCS1 could inhibit GHR and PRLR signaling in Chinese hamster ovary cells, or 293 cells[15,16]. In addition, we also found that cSOCS1b can attenuate cGH/cPRL signaling in Hep G2 cells, though its inhibitory effect seems to be much weaker than that of cSOCS1a (Fig. 4).Our findings provide the first piece of evidence that both SOCS1s can affect PRL/GH signaling in a non-mammalian vertebrate species. Considering the co-expression of cSOCS1a, cSOCS1b, cGHR and cPRLR in all chicken tissues examined here or in an earlier study[22], it is possible to speculate that cSOCS1a, perhaps together with SOCS1b, may negatively regulate GH/PRL signaling in various tissues, thus attenuating the actions of GH/PRL in many physiological processes, such as growth, reproduction and immunity[22,30,31]. Recently, *SOCS1* (*SOCS1a1* in this study) has been implicated in controlling zebrafish growth[32]. Excess GH production in homozygous GH-transgenic zebrafish caused no obvious bodyweight gain at the 23-week stage compared to the non-transgenic fish, but resulted in increased *SOCS1*mRNA levels in the liver, as well as decreased *IGF-1* mRNA levels. This indicates that *SOCS1* expression can be induced by excessive GH and thus inhibit the growth-promoting effect of GH *in vivo*[32]. This important finding from zebrafish also raises a fundamental question whether SOCS1 protein(s) can modify the *in vivo* actions of GH/PRL in chickens, frogs, and mammals, and this possibility is worthy of future research.

5 Conclusions

In the present study, two *SOCS1* genes (*SOCS1a* and *SOCS1b*) were identified in chickens and other non-mammalian vertebrates. Functional studies showed that

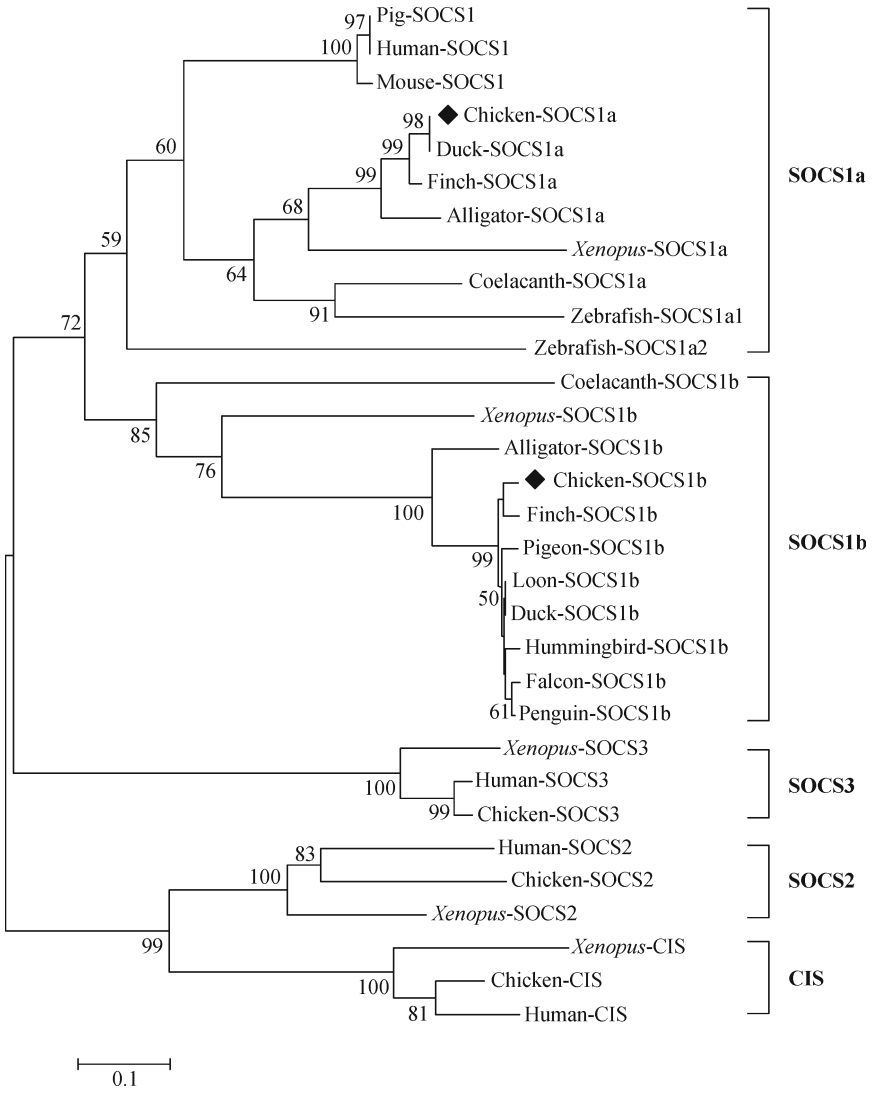

Fig. 5 Phylogenetic tree (constructed by Neighboring-Joining method) showing the evolutionary relationship of *SOCS1a, SOCS1b, SOCS3, SOCS2* and *CIS* genes from non-mammalian and/or mammalian vertebrates. Numbers near each branch point indicate the bootstrap values. The amino acid sequences of all *SOCS or CIS* genes were either retrieved from GenBank or predicted according to genomic sequences.

both cSOCS1 proteins are active and capable of inhibiting cGH/cPRL signaling *in vitro*. These findings, together with the ubiquitous expression of *SOCS1a* and *SOCS1b* in all chicken tissues examined, suggest that cSOCS1a and cSOCS1b may negatively regulate JAK-STAT signaling triggered by cGH/cPRL (perhaps also by many cytokines), and thus affect many physiological processes, such as growth, reproduction and immunity of chickens.

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Compliance with ethics guidelines Xue Xu, Jiannan Zhang, Juan Li and Yajun Wang declare that they have no conflict of interest or financial conflicts to disclose.

All applicable institutional and national guidelines for the care and use of animals were followed.

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