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Molecular characterization and expression analysis of nine CC chemokines in half-smooth tongue sole, *Cynoglossus semilaevis*

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ABSTRACT

Chemokines are a large, diverse group of small cytokines that can be classified into several families, including the CC chemokine family, which plays a pivotal role in host defense by inducing leukocyte chemotaxis under physiological and inflammatory conditions. Here we studied 9 CC chemokines from half-smooth tongue sole (*Cynoglossus semilaevis*). Phylogenetic analysis divided these chemokines into four groups. The tissue specific expression patterns of the 9 chemokines under normal physiological conditions varied much, with most chemokines highly expressed in immune organs, while some other chemokines showing high expression levels in non-immune organs. In addition, the 9 chemokines exhibited similar or distinctly different expression profiles in response to the challenge of virus and intracellular and extracellular bacterial pathogens. These results indicate that in tongue sole, CC chemokines may be involved in different immune responses as homeostatic or inflammatory chemokines.

1. Introduction

Chemokines or chemoattractant cytokines are known as a group of 8–14 kDa molecules that regulate cell migrations under various conditions. They also play roles in normal and pathological processes including allergic responses, infectious and autoimmune disease, angiogenesis, inflammation, and tumor growth and metastasis [1]. Functionally, chemokines are divided into two main categories. Some chemokines are produced and secreted constitutively. These chemokines play roles in immune surveillance and function as homeostatic cytokines [2]. Other chemokines are only produced by cells during infection or following a pro-inflammatory stimulus; these chemokines prompt the migration of leukocytes to an injured or infected site [3]. Such inflammatory chemokines can also activate cells to raise an immune response and commence the wound healing process [4]. Chemokines are structurally related, with most containing four invariant cysteine residues involved in two disulphide bonds [5]. The CC (beta) chemokines comprise a subfamily of the chemokine superfamily and are defined by the arrangement of the first two of four invariant cysteine residues found in all chemokines [6]. In CC chemokines, these two cysteines are adjacent, while in the CXC subfamily of chemokines, they are separated by a single amino acid [7]. Chemokines exhibit promiscuous binding to multiple seven-transmembrane, G-protein coupled CC chemokine receptors [8].

In mammals, the broadest functional classification system divides CC chemokines into inflammatory and homeostatic groups [9,10] based on their expression patterns. This division is widely acknowledged too simplistic. Due to the rapid divergence and independent duplication events within each species, the identification of orthologs became more complicated. In teleost, Peatman and Liu have established CC chemokine classification [11]. Seven large groups of fish CC chemokines have been identified through phylogenetic analysis: the CCL19/21/25 group, the CCL20 group, the CCL27/28 group, the CCL17/22 group, the macrophage inflammatory protein (MIP) group, the monocyte chemotactic protein (MCP) group and a fish-specific group [11].

In teleost, researchers have identified more than double of the chemokines of mammals in zebrafish through analyzing expressed sequence tags (ESTs) and genome sequence [12–14]. This phenomenon may be due to their multiple roles in innate immunity which may be a mechanism to react to various pathogens. These chemokines may work together as a complicated network and coordinate immune responses to specific species [11].

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Table 1
The GenBank accession numbers of CCL family members used for construction of the phylogenetic tree and multiple sequence alignment in this study.

CCL	Accession no.	CCL	Accession no.
Cynoglossus semilaevis		***Danio rerio***	
CCL3a	XP_008309875	CCL2	XP_005162867
CCL3b	XP_008311022	CCL3	XP_003199028
CCL20a	XP_008332070	CCL4	XP_005171406
CCL20b	XP_008306526	CCL5	XP_002666850
CCL20c	XP_008332071	CCL13	XP_001338140
CCL20d	XP_008331981	CCL19	XP_005155699
CCL21	XP_008332797	CCL20	XP_002666702
CCL27a	XP_008308458	CCL21	XP_002661011
CCL27b	XP_008334193	***Rattus norvegicus***	
Homo sapiens		CCL2	P14844
CCL2	P13500	CCL3	P50229
CCL3	P10147	CCL4	P50230
CCL4	P13236	CCL5	P50231
CCL5	P13501	CCL6	Q68FP3
CCL7	P80098	CCL7	Q9QXY8
CCL8	P80075	CCL20	P97884
CCL13	Q99616	***Salmo salar***	
CCL14	Q16627	CCL4	ACI67979
CCL15	Q16663	CCL8	ACI68150
CCL17	Q92583	CCL19	ADM15970
CCL19	Q99731	CCL21	NP_001134739
CCL20	P78556	CCL25	ACI69025
CCL21	O00585	CCL28	NP_001134950
CCL22	O00626	***Pundamilia nyererei***	
CCL23	P55773	CCL3	XP_005754438
CCL24	O00175	***Xiphophorus maculatus***	
CCL25	O15444	CCL3	XP_005817321
Mus musculus		***Oreochromis niloticus***	
CCL2	NP_035463	CCL3	XP_005449343
CCL4	P14097	***Stegastes partitus***	
CCL5	P30882	CCL3	XP_008296008
CCL12	Q62401	***Poecilia reticulata***	
CCL19	O70460	CCL3	XP_008394660
CCL24	Q9JKC0	***Anoplopoma fimbria***	
CCL25	O35903	CCL20	ACQ57955
CCL27	Q9Z1X0	***Takifugu rubripes***	
CCL28	Q9JIL2	CCL13	NP_001266983
Esox lucius		CCL20	NP_001233222
CCL4	ACO14065	***Larimichthys crocea***	
CCL8	NP_001290574	CCL19	NP_001290247
CCL20	ACO13905		
CCL21	NP_001290632		

However, more researches are needed to understand the function of chemokines in fish. Recent studies with cultured fish species have focused on the immunological roles of chemokines in the defense against pathogens [15]. To date, identification and functional analyses of CC chemokines have been carried out with rainbow trout [16–21], carp [22], catfish [23–25], Japanese flounder [26–29], turbot [30], and half-smooth tongue sole [31–33]. The identification of CC chemokines in teleost was often through analyzing EST database and bioinformatics method, as genome sequences of many cultured fish were released. For example, 32 distinct CC chemokines were identified by analysis of EST database in Atlantic cod (*Gadus morhua*) [34]. In rainbow trout, several chemokines were identified, which were regulated in expression by viral hemorrhagic septicemia virus and infectious pancreatic necrosis virus [16–18,21].

Recently, the genome sequence of tongue sole has been completed [35], which revealed the existence of 11 putative CC chemokine genes. The aim of this study was to examine, in a comparative and systematic manner, the expression profiles of tongue sole CC chemokines. For this purpose, we selected nine of these putative CCLs that could be successfully amplified by PCR and had not been studied previously. To promote the use of standard nomenclature, we re-named these CCLs based on our phylogenetic analysis. The nine CCLs analyzed in this study were named CCL3a, CCL3b, CCL20a, CCL20b, CCL20c, CCL20d, CCL21, CCL27a and CCL27b, which were originally named CCL3, CCL4, CCL26, CCL20, CCL20, CCL20, CCL20, CCL13, and CCL20, respectively [35]. The

Table 2
Primers used in this study.

Primers	Sequences (5′–3′)
CCL20a-RT-F	GTGCTGCACACAGTACAATGA
CCL20a-RT-R	TGCACCCACTTTGAGTTAGG
CCL20b-RT-F	CCATCGTTTTCCGGTGGAGA
CCL20b-RT-R	TTCCTGGACGTGCCGTTATG
CCL20c-RT-F	TGTGTCCCATCAATGCCATCA
CCL20c-RT-R	TGTTTGACTGGGCTTCAGTGT
CCL20d-RT-F	GACGAGTGGGTGAGAAACACT
CCL20d-RT-R	CAGTGTCTGTGGTGCTGAAGA
CCL27b-RT-F	CAGACTGCAGCATACAAGCC
CCL27b-RT-R	AGCCACATGGTTCGGTGAG
CCL3a-RT-F	GGAGAACGTGGTCAGCTACA
CCL3a-RT-R	CCCAGGTGGCTGAAGGTCTA
CCL3b-RT-F	CTTGCCTGTGAAGAGGGTGAT
CCL3b-RT-R	TGGATCGGCACAGATTTTCCT
CCL27a-RT-F	TCCATTTGCTGCTTCACACG
CCL27a-RT-R	AGTCATTCTGGCGTGCAACA
CCL21-RT-F	CTGGCCCAAGTGTCCTACG
CCL21-RT-R	GAATGTTACAGCCTCCGTCCA