Cloning and Characterization of Grass Carp (Ctenopharyngodon idella) IL-23 Receptor

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Abstract. IL-23 receptor (IL-23R), one member of IL-23 receptor complex family, is expressed by both T cells and NK cells, including NKL cells. It can bind to IL-23 and activate the Jak-Stat signaling cascade. Soluble IL-23R can block IL-23 signal by reducing the RORγt expression. In this study, we isolated and identified the cDNA sequence of grass carp IL-23R (gcIL-23R). By using the bioinformatics approaches, we analysis the cDNA of grass carp IL-23R which encodes a peptide of 862 amino acids and is 30-78% identities to its homologs in other fish species. Moreover, zebrafish and grass carp IL-23R shared relatively low amino acid identities with human homolog. Further gene organization analysis showed gcIL-23R possesses five extra exons and an additional FN3 Superfamily domain compared with human counterpart. These data facilitate our understanding of IL-23R involving in fish immunity.

Introduction

As a new member of Interleukin-12 cytokine family, IL-23 is a heterodimeric cytokine and is composed of the IL-12p40 subunit with a novel cytokine-like subunit p19, which plays an important role in stimulating particular populations of memory T cell and has a potential antitumor activity [1]. IL-23 receptor complex is also composed of two chains: the IL-12 receptor β1 chain and the IL-23 receptor chain [2]. When IL-23 binds to its receptor complex, it triggers a series of chemical signals to T helper cells and mediates its proinflammatory effects through the activation of Th17 cells that secrete IL-17 [3, 4]. Knockout mice deficient in either p19 or p19 receptor (IL-23R) is resistant to experimental autoimmune encephalomyelitis (EAE) [5], collagen-induced arthritis (CIA), and inflammation bowel disease (IBD), highlighting the importance of IL-23/IL-23R in the inflammatory pathway [6]. Recently, several reports have established that IL-23R deficiency will cause Th17 development stall at the early activation stage and then lead to less Th17 proliferation and fewer effector Th17 cells [7]. The exogenous soluble human IL23R protein could bind to human/mouse IL-23 complex and inhibit the binding of mouse IL-23 to endogenic mouse IL-23 receptor complex, therefore lowering the IL-17/IL-22 expression [8]. In the present study, we described the cloning and characterization of gcIL-23R, and compared its structure with those of mammalian counterparts.
Materials and Methods

Animals

Chinese grass carp, with body weight of about 0.75-1.0 kg were obtained from Chengdu Tongwei Aquatic Science and Technology Company (Chengdu, China) and were maintained in laboratory tanks with room temperature for 2 weeks before experimental processing. The head kidney leukocytes isolation was taken from freshly killed fish according to the Regulation of Animal Use in Sichuan province, China.

Molecular Cloning of gcIL-23R

Total RNA was extracted from grass carp intestine by using TRIzol Reagent (Invitrogen, Carlsbad, CA) and then reverse transcribed to cDNA using Superscript II reverse transcriptase (Invitrogen) and using oligo d(T)\textsubscript{18} as the primer. The newly synthesized cDNA was served as the template for cloning gcIL-23R partial sequence. We obtained partial sequences based on the conserved regions of IL-23R sequences of other fishes. The target PCR fragments were subcloned into pGEM-T easy vector (Promega) and the selected clones were sequenced. Based on the partial sequence, the full-length cDNA sequences were gotten using RACE (Life Technology) according to the manufacturer’s instructions.

Sequence Analysis of gcIL-23R

The full-length sequence of gcIL-23R cDNA was analyzed by using the BLAST program in NCBI (http://www.ncbi.nlm.nih.gov). The deduced amino acid sequence of gcIL-23R was analyzed using the ExPASy (http://www.expasy.org/tools/). The signal peptide and the transmembrane region were predicted by using the SignalP4.1 (http://www.cbs.dtu.dk/services/SignalP/) and TMHMM2.0 Server (http://www.cbs.dtu.dk/services/TMHMM), respectively. The multiple sequence alignments were generated by using DNAMAN software (Lynnon Biosoft, Pointe-Claire, Canada). Phylogenetic trees were constructed on the basis of amino acid differences (p-distance) using the neighbor joining algorithm (complete deletion) in MEGA Version 2.1.

Results

Sequence Analysis of Grass Carp IL-23R

The full-length grass carp IL-23R cDNA sequence with 2589 bp encoded a 862-aa protein. The deduced gcIL-23R protein with molecular weight of 96.65 kDa and possessed a signal peptide of 23 amino acids, and a theoretical pI of 6.31. Two potential N-glycosylation sites were predicted in gcIL-23R (position 153 and 231) (Fig. 1).
Figure 1. Nucleotide and deduced amino acid sequences of gcIL-23R. The signal peptide was indicated with a black line. The potential N-glycosylation sites is boxed.

Amino Acid Sequence and Phylogenetic Analysis of gcIL-23R

Amino acid alignment showed that 78.47% and 52.66% nucleotide identities of gcIL-23R to its homologs in common carp and zebrafish, respectively, and displayed lower amino acid identities (13-15%) with that from other non-piscine (Table 1).

Table 1. Amino acid and nucleotide identities of grass carp IL-23R with the known IL-23R sequences in other vertebrates.

<table>
<thead>
<tr>
<th>Identity</th>
<th>Common carp (%)</th>
<th>Zebrafish (%)</th>
<th>Rainbow trout (%)</th>
<th>Sheep (%)</th>
<th>Cattle (%)</th>
<th>Mouse (%)</th>
<th>Human (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass carp</td>
<td>78.47</td>
<td>52.66</td>
<td>31.77</td>
<td>13.23</td>
<td>12.88</td>
<td>14.48</td>
<td>15.03</td>
</tr>
<tr>
<td>(amino acid)</td>
<td>(nucleotide)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass carp</td>
<td>85.78</td>
<td>79.69</td>
<td>40.09</td>
<td>29.86</td>
<td>29.9</td>
<td>30.76</td>
<td>30.42</td>
</tr>
</tbody>
</table>

The structure of the IL-23R demonstrated that it is typical of a receptor molecule, consisting of an extracellular region of 606 amino acids and a cytoplasmic region of 232 amino acids, bisected by a transmembrane region of 24 amino acids by using TMHMM2.0 Server and SignalP 4.1 server analysis (data not shown). Multiple sequence alignment showed gcIL23R possessed some conserved cysteine residues and an FN3 superfamily domain (Fig. 2A). Phylogenetic analysis showed gcIL-23R was closely related to the fish and was distant from its homologs in mammalian species (Fig. 2B).
Comparison of gcIL-23R Gene Organization with Mammalian Homology

Inspection of the genome of IL-23R genes structure revealed that gcIL-23R gene possessed five extra exons compared to human homology (and extra four ones compared to mouse IL-23R). More precisely, the gcIL-23R gene possessed 15 exons/14 introns, human IL-23R
gene possessed 10 exons/9 introns and mouse IL-23R gene possessed 11 exons/10 introns. Grass carp IL-23R have two FN3 Superfamily domains while only one was present in mammalian homology (Fig. 3).

Figure 3. Schematic representation of gcIL-23R gene structure along with human and mouse homology. Boxes are exons and horizontal lines are introns. Values in boxes represent the number of nucleotides. FN3 Superfamily domains are indicated above the exon.

Discussion

Th17 cells belong to a novel T helper cells subset that mainly expresses IL-17 during inflammatory responses[9]. Th17 differentiation can be induced by IL-6 and TGF-β, and differentiated Th17 cells are further stabilized and amplified by the actions of IL-23[10]. Previous studies have indicated that IL23R-CHR, a truncated IL-23R extracellular domain, is able to bind IL-23 in vitro and capable of blocking IL-23/IL-17 pathway and suppressing IL-23-mediated production of IL-17A through a STAT3-RORγ pathway in vitro. In the present study, the gcIL-23R sequence was isolated and characterized (Fig.1). The characterization of gcIL-23R was clearly supported by the conservation of important structural residues, phylogenetic analysis and gene organization. According to the current alignment, it showed that fish IL-23R genes formed a cluster distinct from other members and gcIL-23R had a higher conservation with zebrafish and common carp compared with rainbow trout (Fig. 2). Domains homologous to FN3 repeats have been found in various animal protein families including other extracellular-matrix molecules, cell-surface receptors, enzymes, and muscle proteins. FN3 domains are thought to mediate protein-protein interactions and to act as spacers [11]. Comparison of the gene structure of the IL-23R genes revealed that gcIL-23R possesses five extra exons and an additional FN3 Superfamily domain compared to human homology. Interestingly, 2-6 exons of gcIL-23R have similar size with 2-5 exons of human IL-23R. We hypothesize that the existence of FN3 domain in exon 9-12 with functional redundancy in teleost may be expressed selectively during evolution.

In summary, we report the cloning and characterization of grass carp orthologous of the mammalian IL-23R subunit. It will provide clues toward to explore the fundamental mechanisms of IL-23R involving in adaptive immunity in fish. Future studies of recombinant expression and purification of soluble gcIL-23R will be done, thereby directly revealing the immunological role of soluble gcIL-23R in grass carp immunity.
References


