Molecular Characterization of Caspase8 in Grass Carp (Ctenopharyngodon Idella)

Sheng-Nan ZHANG\(^a\), Ning GAN, Na ZHANG and Hong ZHOU\(^b, *\)

School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, People's Republic of China

\(^a\)zhangshengnan0824@163.com, \(^b\)zhouhongzh@uestc.edu.cn

*Corresponding author

Keywords: Caspase8, Molecular characterization, Gene structure, Grass carp.

Abstract. Caspase8 is a crucial effector member of the caspases family. It is well known that caspase8 plays a vital role in apoptosis signal transduction mediated through death receptor pathway in mammals. In the present study, grass carp caspase8 (gcCasp8) coding sequence was isolated and identified. The gcCasp8 CDS and amino acid sequence shared 45.36%-80.06% and 32.5%-71.25% with its counterparts in other teleost species and mammals, respectively. Furthermore, similar to human counterpart, the gcCasp8 CDS consisted of 8 exons and 7 introns, with one exon less than zebrafish homolog. In addition, gcCasp8 possessed caspase domain and catalytic site conserved with homologs in the known species. These results show that grass carp caspase8 has a structure similarity to teleost and mammalian caspase8 orthologs, and our study lay the theoretically foundation for further study of caspase8 function involving in apoptosis in grass carp.

Introduction

Caspases (cysteinyl aspartate specific proteinase) are a group of endoproteases [1]. They can hydrolyze peptide bonds depends on the catalytic cysteine residues in the active site and regulate cell growth, differentiation, inflammation and apoptosis consequently [2-4]. Caspases can be classified into two groups based on their functions: one group relevant to inflammation and another one involving in apoptosis [1, 4, 5]. The apoptotic group can be divided into initiator caspases and effector caspases according to their roles [3].

Caspase8, one member of the apoptosis effector caspases, is activated by upstream caspase cleavage in a cascade manner and implemented apoptosis in mammals [3, 5, 6]. It is well known that cell surface “death receptors” such as TNFR1 and Fas mediate the caspase8-cascaded apoptosis (extrinsic pathway) [6]. However, caspase8 cannot regulate apoptosis through the mitochondrial or Bcl-2-regulated (intrinsic) pathway [7].

In vertebrates, most caspase homologs have been identified in mammal and teleost [8-10]. According to their structure analysis, caspase8 has an extended amino-terminal prodomain named death effector domain (DED) and a CASc domain (carboxyl-terminal catalytic domain), similar to caspase10 and different from other caspase homologs including caspase1 and caspase3. The caspases have been identified in various fish, including zebrafish [10, 11], sea bass [12], rock bream [13], and striped murrel [14]. In this study, we isolated and identified the caspase8 from grass carp. The analysis of gcCasp8 CDS revealed that it was similar to homologs in mammal and teleost. Structure analysis of gcCasp8 showed that it possessed conserved prodomain and CASc domain. This work will facilitate a better understanding of the involvement of caspase8 in fish apoptosis.
Materials and Methods

Fish

Chinese grass carp (average 1000 g) were purchased from Chengdu Tongwei Aquatic Science and Technology Company (Chengdu, China). The fish were acclimatized in the laboratory for two weeks and kept in aerated seawater at 20 ± 2°C prior to experimental processing as reported previously [15]. The fish were fed daily with dry food from Tongwei Aquatic Science and Technology Company. All experiments complied with the Regulation of Animal Use in Sichuan Province, China.

Molecular Cloning of gcCaspase8

Total RNA was extracted from the head kidney of grass carp by using TriPure Isolation Reagent (Roche). The first strand cDNA was synthesized from the isolated total RNA adopting the Superscript III RT-PCR system (Promega) according to the manufacturer’s protocol. The cDNA sequences of gcCasp8 were obtained by PCR using high fidelity Taq (NEB) and gene-specific primers, designed based on the available sequences of other fish caspase8. Then the full cDNA sequence of gcCasp8 was sequenced.

In Silico Analysis of gcCasp8

Homologues analysis was performed using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) at the National Center for Biotechnology Information (NCBI). The deduced amino acid sequence of gcCasp8 was analyzed using Translate tool in the ExPASy Molecular Biology server (http://web.expasy.org/translate/). The signal peptide was predicted by using the SignalP4.1 (http://www.cbs.dtu.dk/services/SignalP/). The domains and motifs of gcCasp8 were analyzed using SMART program (http://smart.embl-heidelberg.de/) and protein BLAST at NCBI. In order to find out the evolutionary conserved residues of gcCasp8, multiple sequence alignment was created with DNAMAN. And a phylogenetic tree of gcCasp8 was developed using MEGA 4 to obtain its evolutionary relationship, respectively.

Results

Characterization of gcCasp8 Gene

We searched for grass carp counterpart based on other fish and mammalian caspase8 in the GenBank database, and cloned and obtained the full-length coding sequence of gcCasp8 with 1413bp coding 470 amino acids (aa) (Fig. 1A). The deduced gcCasp8 was a 54.03 kDa polypeptide with a theoretical pl of 5.25. The amino acid sequence of gcCasp8 shared 65.43% and 71.25% identities with zebrafish and common carp homologs respectively and shared relatively low identities (37.42%-39.88%) to its counterparts in other vertebrates (Table 1).
Figure 1. A: Nucleotide and deduced amino acid sequences of grass carp caspase8. The ORF is shown in upper case. The nucleotides in bold indicate the start codon (ATG) and the stop codon (TGA). The putative amino acid sequence is shown under the triplet codon. The death effector domains are indicated with underline, the putative CASc region is indicated with a double-crossed. The predicted N-glycosylated site was in bold and circled with a black box. B: An unrooted phylogenetic tree is constructed by the neighbor joining method by MEGA 4 software using the amino acid sequences of caspase8 from various vertebrates. The numbers indicate the bootstrap confidence values obtained for each node after 1000 replications. The scale on the bottom refers to the percentage divergence (p-distance). The grass carp caspase8 was in black box. GenBank accession numbers of caspase8 protein in this analysis: Human: AH007578; Mouse: BC049955; Cattle: BT030512; Chicken: AY057939; Frog: NM_001085565; Snakehead murrel: HF913727; Rainbow trout: HE608242; Zebrafish: NM_131510; Common carp: KC822471.

To reveal the evolutionary relationship of gcCasp8 with its homologs in other species, an unrooted phylogenetic tree was constructed using the neighbor joining method by MEGA 4 software. As shown in Fig. 1B, all teleost caspase8 were grouped and separated from the mammalian caspase8 clade. Moreover, gcCasp8 grouped in the clade of zebrafish and common carp caspase8.

Table 1. Amino acid and nucleotide identities of grass carp caspase8 with the known caspase8 sequences in other vertebrates.

<table>
<thead>
<tr>
<th>Identity</th>
<th>Common carp (%)</th>
<th>Zebrafish (%)</th>
<th>Snakehead murrel (%)</th>
<th>Rainbow trout (%)</th>
<th>Frog (%)</th>
<th>Chicken (%)</th>
<th>Cattle (%)</th>
<th>Mouse (%)</th>
<th>Human (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass carp (amino acid)</td>
<td>71.25</td>
<td>68.46</td>
<td>41.1</td>
<td>44.34</td>
<td>32.5</td>
<td>37.42</td>
<td>38.71</td>
<td>39.88</td>
<td>39.8</td>
</tr>
<tr>
<td>Grass carp (nucleotide)</td>
<td>80.66</td>
<td>75.21</td>
<td>53.18</td>
<td>52.97</td>
<td>45.36</td>
<td>51.67</td>
<td>50.33</td>
<td>52.01</td>
<td>51.4</td>
</tr>
</tbody>
</table>
Amino Acid Sequence Analysis of gcCasp8

To reveal the domain structure of gcCasp8, we used the online program SMART and protein BLAST to forecast the probable motifs according to mammalian caspase protein database. We found that gcCasp8 consisted of two conserved death effector domain (DED) and one carboxyl-terminal catalytic domain (CASc, interleukin 1 beta converting enzyme (ICE) homologues). And two conversed regions essential for catalytic activity were discovered in gcCasp8. Although there was no signal peptide, we predicted one potential N-glycosylation motif in its sequence (Fig. 2).

Figure 2. Multiple alignment of the deduced amino acid sequence of gccaspase8 with the known caspase8 homologs in other vertebrates. The deduced death effector domains were indicated by the upper black line and predicted CASc region was showed with a gray rectangle box. Fully conserved residues are noted by the lower cases below and the predicted conversed regions essential for catalytic activity is indicated with a black rectangle box. The potential N-glycosylation motif is showed with a black box.

Genomic Organization of Gccaspasse8 CDS

To distinguish intron-exon boundaries, we compared the gcCasp8 CDS by carrying out off-line BLAST on grass carp genome sequence. The genomic organization of gcCasp8 CDS was examined and compared it with that of the mammalian caspase8 sequences. As shown in Fig. 3, the gcCasp8 CDS consisted of 8 exons and 7 introns, similar with the human counterpart. However, by searching the GenBank genome database, we found the CDS of zebrafish caspase8, composed of 9 exons and 10 introns, have one more exon than gcCasp8.

Figure 3. Schematic representation of the genomic organization of caspase8 (casp8) CDS from grass carp. Black boxes indicated the coding regions (exons) and black line revealed non-coding regions (introns), respectively. The numbers in boxes showed the sequence length of exons.
Discussion

Caspase8, a member of caspases superfamily, plays a crucial role in programmed cell death when activated by upstream death receptors [5, 6, 16]. In consideration of vital function of caspases, caspase8 gene has only been identified in a few fish species [10, 14]. Based on computational genomic structural analyses, we isolated the putative caspase8 coding sequence from grass carp head kidney (Fig. 1A). Homologues analysis using BLAST online program demonstrated that gcCasp8 CDS shared high conservation of nucleotide and amino acid sequence with zebrafish and common carp homologs (Table 1). Gene organisation analysis showed that caspae8 CDS consist of 9 exons and 8 introns in mouse and zebrafish, however, gcCasp8 possessed 8 exons and 7 introns, more similar to human caspase8 (Fig. 3). This phenomenon may be accounted for the diversity in different fish species during evolution [10]. According to amino acid sequence alignment, the unrooted phylogenetic tree was constructed and results showed that gcCasp8 grouped with zebrafish and common carp counterparts, and indicated gcCasp8 was closer with zebrafish and common carp homologs during evolution. Although gcCasp8 shared relatively low nucleotide and amino acid identity with mammalian counterparts, the residues critical for catalytic activity were conserved in both mammal and teleost. Meanwhile, gcCasp8 composed of two conserved death effector domain (DED) in N-terminus and one carboxyl-terminal catalytic domain CASc. The functional domains of caspase8 protein were relatively high conserved in both teleost and mammals (Fig. 2). Actually, zebrafish caspse8 shows its conserved role involving in apoptosis [10], indicating the evolutionary conservation of caspase8 functionality.

Caspases were important proteinase involved in inflammation and apoptosis. Generally, caspase8 was a crucial effector for downstream signal transduction of apoptosis in vertebrates. Given that several other genes involved in apoptosis were identified in a few fish [14, 17], such as zebrafish and murrel, the characterization of caspase8 in grass carp appears to be particularly important. This study will provide new insights into the information of fish caspases, and an important theoretical basic for subsequently clarifying the role of caspase8 in fish.

References


