Gene 463 (2010) 21–28

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

The evolution of somatostatin in vertebrates

Yun Liu [a,1], Danqi Lu [a,1], Yong Zhang [a], Shuisheng Li [a], Xiaochun Liu [a,*], Haoran Lin [a,b,*]

[a] State Key Laboratory of Biocontrol, Institute of Aquatic Economic Animals, and the Guangdong Province Key Laboratory for Aquatic Economic Animals, Sun Yat-Sen University, Guangzhou 510275, China
[b] College of Ocean, Hainan University, Haikou 570228, China

ARTICLE INFO

Article history:
Received 21 January 2010
Received in revised form 23 April 2010
Accepted 29 April 2010
Available online 21 May 2010

Received by J.G. Zhang

Keywords:
Somatostatin
Gene duplication
Comparative genomics
Evolution
Peptide hormone

ABSTRACT

Somatostatins (SS) play important roles in the regulation of growth in vertebrates. In the present study, we identified six SS genes in zebrafish and named them *SS1*, *SS2*, *SS3*, *SS4*, *SS5* and *SS6*. We subsequently found that five SS genes (*SS1*, *SS2*, *SS3*, *SS4* and *SS5*) also existed in stickleback, medaka, *Takifugu* and *Tetraodon*. Phylogenetic analysis showed that vertebrate SS genes were grouped into five clades. Using a comparative genomic approach, we further investigated the evolutionary origin of these SS genes in vertebrates, and the results revealed that: (1) *SS1*, *SS2* and *SS5* were generated by two rounds of genome duplications (2R) that happened during the early stages of vertebrate evolution; (2) *SS4* is an *SS1* paralog generated by a third genome duplication (3R) that occurred to most teleost fish; and (3) *SS3* and *SS6* were produced by tandem duplication of *SS1* and *SS2* in teleost fish. RT-PCR analysis revealed that all six SS genes were functionally expressed in different zebrafish tissues. These data indicate that both genome-wide duplication and local duplication contribute to the expansion of SS genes in vertebrates.

1. Introduction

Somatostatins (SS) play central roles in the negative control of growth hormone secretion in the pituitary (Brazeau et al., 1973; Klein and Sheridan 2008; Gahete et al., 2009). Four SS genes have been reported in vertebrates so far (Tostivint et al., 2008). The *somatostatin 1* (*SS1*) has been characterized in all vertebrates from agnathans to mammals (Brazeau et al., 1973; Andrews et al., 1988). The *somatostatin 2* (*SS2*) has been reported in many bony fish species (Nelson and Sheridan 2005; Tostivint et al., 2008). Also, a partial genomic sequence encoding the putative *SS2* has been identified in the elephant shark (*Callorhinchus milii*) genome database (Tostivint et al., 2008). *Somatostatin 3* (*SS3*) (previously termed SSII) was identified in many teleost fish (Conlon et al., 1988; Moore et al., 1995; Lin et al., 1999; Ye et al., 2005) whereas *somatostatin 4* (*SS4*) (previously termed atypical SSII gene) was only found in three teleost fish species belonging to the ostariophysi (Magazin et al., 1982; Ferraz de Lima et al., 1999; Devos et al., 2002). Several forms of mature somatostatin peptides have been characterized in vertebrates, and all of them contain two conserved Cys residues, forming a cyclic secondary structure (Veber et al., 1979; Tostivint et al., 2008). Two forms of mature peptides (SS-14 and SS-28) can be produced by the processing of prepro-SS1 in different tissues in mammals and some teleost fish (Nelson and Sheridan 2005; Tostivint et al., 2008). The prepro-SS2-derived peptide contains a Pro^2 residue that is absent in other SS peptides (Spier and de Lecea, 2000). Other forms of somatostatin peptides derived from prepro-SS3 and prepro-SS4 have also been identified in teleost fish (Nelson and Sheridan 2005; Tostivint et al., 2008). All of these mature SS peptides are located at the C-terminal of SS precursors (Tostivint et al., 2008).

Comparative genomics not only provides us unprecedented opportunities to discover new peptide hormone genes (Lee et al., 2007; Huang et al., 2009) but also the opportunity to investigate the evolutionary history of peptide hormone gene families (Conlon and Larhammar 2005; Lee et al., 2007; Sundström et al., 2008). Many vertebrate peptide hormone genes are members of a certain gene family (Conlon and Larhammar 2005) that is composed of related genes possibly descending from a common ancestor. Gene duplication is the primary force producing multigene families (Ohno 1970; Zhang 2003; Van de Peer, 2004). Two rounds of genome duplications (2R) occurred in the early stages of vertebrate evolution (Amores et al., 1998; Dehal and Boore 2005; Kasahara et al., 2007), before the separation of cartilaginous fish and bony fish (Venkatesh et al., 2007; Ravi et al., 2009). A third round of genome duplication (3R) occurs in most extant teleost fish (Jaillon et al., 2004; Meyer and Van de Peer 2005). Local duplications of individual gene have also happened (Van

Abbreviations: SS, somatostatin; 2R, two rounds of genome duplications; 3R, three rounds of genome duplications; UII, urotensin II; URP, urotensin related peptide; NJ, neighbor-joining; TP53, tumor protein 53; DVL, disheveled.

* Corresponding authors. X. Liu is to be contacted at tel.: +86 20 84112511; fax: +86 20 84113717. H. Lin, State Key Laboratory of Biocontrol, Institute of Aquatic Economic Animals, and the Guangdong Province Key Laboratory for Aquatic Economic Animals, Sun Yat-Sen University, Guangzhou 510275, China. Tel: +86 20 84113791; fax: +86 20 84113717.

E-mail addresses: lsslxc@mail.sysu.edu.cn (X. Liu), lsslhr@mail.sysu.edu.cn (H. Lin).

[1] These authors contributed equally to this work.

0378-1119/$ – see front matter.
doi:10.1016/j.gene.2010.04.016

de Peer, 2004). Both genome-wide duplications and local gene duplications are known to be involved in the evolution of peptide hormone families (Conlon and Larhammar 2005; Tostivint et al., 2006).

Although the physiological functions of SS have been intensively studied, little attention has been paid to the evolution of this multigene family. To date, few comparative genomic studies have been carried out. By synteny analysis, a recent study confirmed that teleost *SS2* is orthologous to mammalian *SS2* (Tostivint et al., 2004). More recent data revealed that *SS1* and *urotensin II* (UII) on one hand, *SS2* and *urotensin related peptide* (URP) on the other hand, might belong to the same gene family that has been expanded by ancient genome duplications (Tostivint et al., 2006). In teleost fish, *SS3* and *SS1* are arranged in tandem, indicating that *SS3* was generated by local duplication of *SS1* (Tostivint et al., 2008). Tostivint et al. recently reviewed the above knowledge of SS/UII supergene family and propose a hypothesis on their evolutionary history (2008). However, the evolutionary origin of *SS4* is not clear (Tostivint et al., 2004, 2008). Moreover, it remains unknown whether other types of SS genes exist. The aims of the present study are to discover new SS genes and to reveal the evolutionary history of multiple SS genes in vertebrates.

2. Materials and methods

2.1. Animals

Zebrafish were obtained from a local fish market in Guangzhou, China. All animal experiments were conducted in accordance with the guidelines and approval of the Animal Research and Ethics Committees of the Sun Yat-Sen University.

2.2. Data mining

To identify SS genes in different species, tblastn was used to search the genome databases of human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), western clawed frog (*Xenopus tropicalis*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), stickleback (*Gasterosteus aculeatus*), spotted green pufferfish (*Tetraodon nigroviridis*), fugu (*Takifugu rubripes*) and sea squirts (*Ciona intestinalis* and *Ciona savignyi*) available in the Ensembl database (http://www.ensembl.org). The version of the genome databases is Ensembl release 56. We also searched the genome database of the elephant shark (*C. milii*) at http://esharkgenome.imcb.a-star.edu.sg, the genome database of the Florida lancelet (*Branchiostoma floridae*) at http://genome.jgi-psf.org and that of the sea lamprey (*Petromyzon marinus*) at http://pre.ensembl.org. The conserved SS mature peptides from both teleost fish and tetrapods were used as the query sequences. Genomic regions containing putative new SS genes were downloaded and ORFs were predicted by Genscan available at http://genes.mit.edu/GENSCAN.html. The tblastn search of the EST database was performed at http://blast.ncbi.nlm.nih.gov.

2.3. Molecular cloning of zebrafish SS3, SS5 and SS6 cDNAs

Total RNA from mixed zebrafish tissues was prepared using Trizol (Invitrogen, USA). One microgram of isolated RNA was used to synthesize the first-strand cDNA using the ReverTra Ace-α First-strand cDNA Synthesis Kit (TOYOBO, Japan). Gene-specific primers flanking open reading frames (ORFs) of zebrafish *SS3*, *SS5* and *SS6* were designed according to the predicted sequences. All primers used in the present study are listed in Table S1.

For PCR reactions in this study, amplifications were performed with an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 15 s, 52–58 °C for 15 s and 72 °C for 1–1.5 min. The reaction was ended by a further extension of 10 min at 72 °C.

The amplification products were separated by agarose gel electrophoresis, and a band of the desired size was excised and purified using the E.Z.N.A. Gel Extraction Kit (Omega BioTek, USA). The purified amplification products were subcloned into the pTZ57R/T vector (Fermentas, USA) and sequenced on an ABI 3700 sequencer (Applied Biosystems, USA). Three different positive clones were sequenced to confirm the sequence information.

2.4. Phylogenetic analysis

SS sequences were aligned with Clustal X 1.81. A phylogenetic tree was constructed with MEGA 3.1 using neighbor-joining (NJ) with default settings. One thousand bootstrap replications were conducted. The bootstrap values lower than 50 were removed.

2.5. Genomic synteny analysis

Genomic synteny analysis was performed at Ensembl genome databases based on Ensembl release 56. The identified SS genes are located at four different chromosomes in the genomes of stickleback, zebrafish, medaka, *Takifugu* and *Tetraodon*. Genes around each stickleback SS gene were used to search for orthologs in the genome databases of zebrafish and human. The identified zebrafish and human orthologs retained within a certain syntenic block are shown in the synteny map whereas lost or trans-located gene orthologs are shown in the supporting information. To identify paralogous gene pairs of the three human chromosomal regions (1p36/3q28/17p13.1), about 10 Mb of each chromosomal fragment was analyzed. Detailed chromosomal locations of gene orthologs and paralogs can be found in the Supporting information.

2.6. Tissue expression patterns of zebrafish SS1, SS2, SS3, SS4, SS5 and SS6

The expression profiles of six SS genes in zebrafish were examined by RT-PCR using gene-specific primers flanking the ORF of each SS transcript (see Table S1 for primer information). The cDNA templates of the telencephalon, optic tectum thalamus, hindbrain, hypothalamus, gill, heart, liver, spleen, intestine and ovary were prepared as described above. Mock RT reactions without reverse transcriptase were used as negative controls for the reactions. *18S* was amplified in parallel to verify the integrity of all cDNA samples. Five microliters of each reaction product was separated on a 1.5% agarose gel containing ethidium bromide and visualized on a Gel Doc System (Bio-Rad, USA). No contamination was found, as all the negative controls gave no amplified products.

3. Results and discussion

3.1. Identification of SS genes in vertebrates

To identify SS genes, we searched the genome databases of different vertebrates and three invertebrates. New SS genes have been discovered in teleost fish. Six SS gene candidates were identified from the zebrafish genome database. Among them, *SS1*, *SS2*, *SS3* and *SS4* were reported previously (Devos et al., 2002; Tostivint et al., 2008), but *somatostatin 5* (SS5) and *somatostatin 6* (SS6) are newly discovered. Because zebrafish SS3, SS5 and SS6 cDNA sequences have not yet been confirmed by experiments, we cloned and sequenced them (Fig. S1). Similar with the known SS precursors, the newly described SS5 and SS6 precursors also contain the mature peptides (SS-14) at their C-terminal (Fig. S1). A disulfide linkage could be formed by two conserved Cys residues in these mature peptides.

Five SS genes (excluding *SS6*) were also identified in the other genome-sequenced teleost fish, including stickleback, medaka,