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Fish genomes provide novel insights into the evolution of vertebrate secretin receptors and their ligand

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ABSTRACT

The secretin receptor (SCTR) is a member of Class 2 subfamily B1 GPCRs and part of the PAC_1/VPAC receptor subfamily. This receptor has long been known in mammals but has only recently been identified in other vertebrates including teleosts, from which it was previously considered to be absent. The ligand for SCTR in mammals is secretin (SCT), an important gastrointestinal peptide, which in teleosts has not yet been isolated, or the gene identified. This study revises the evolutionary model previously proposed for the secretin-GPCRs in metazoan by analysing in detail the fishes, the most successful of the extant vertebrates. All the *Actinopterygii* genomes analysed and the *Chondrichthyes* and *Sarcopterygii* fish possess a SCTR gene that shares conserved sequence, structure and synteny with the tetrapod homologue. Phylogenetic clustering and gene environment comparisons revealed that fish and tetrapod SCTR shared a common origin and diverged early from the PAC_1/VPAC subfamily group. In teleosts SCTR duplicated as a result of the fish specific whole genome duplication but in all the teleost genomes analysed, with the exception of tilapia (*Oreochromis niloticus*), one of the duplicates was lost. The function of SCTR in teleosts is unknown but quantitative PCR revealed that in both sea bass (*Dicentrarchus labrax*) and tilapia (*Oreochromis mossambicus*) transcript abundance is high in the gastrointestinal tract suggesting it may intervene in similar processes to those in mammals. In contrast, no gene encoding the ligand SCT was identified in the ray-finned fishes (*Actinopterygii*) although it was present in the coelacanth (lobe finned fish, *Sarcopterygii*) and in the elephant shark (holocephalian). The genes in linkage with SCT in tetrapods and coelacanth were also identified in ray-finned fishes supporting the idea that it was lost from their genome. At present SCTR remains an orphan receptor in ray-finned fishes and it will be of interest in the future to establish why SCT was lost and which ligand substitutes for it so that full characterization of the receptor can occur.

1. Introduction

Secretin (SCT) was identified over 100 years ago in the ground-breaking experiments of Bayliss and Starling that revealed it was a regulator of exocrine pancreatic secretion (1902) (Bayliss and Starling, 1902). This heralded a new era in the study of regulatory systems and the term "hormone" was adopted for chemical messengers produced in one organ, released into the circulation and acting on a distant target organ (Starling, 1905). In 1981, Jensen and Gardner identified a receptor for SCT (SCTR) in the human pancreas and in 1991 it was cloned from the rat NG108-15 cell line (Ishihara et al., 1991). SCTR is a member of the Class 2 B1 subfamily of G-Protein Coupled Receptors (GPCRs), which is a group of seven helical cell transmembrane receptors.

In mammals, SCT is a 27 amino acid peptide and is involved in many functions (Lam et al., 2008). In the brain it is a potent neuropeptide and controls the secretion of several neuropeptides (Yamagata et al., 2008; Chu et al., 2009; Velmurugan et al., 2010) and in the periphery it regulates body water homeostasis (Chu et al., 2007, 2009), relaxes vascular smooth muscle (Fara and Madden, 1975; Bell and McDermott, 1994) and enhances secretion of reproductive hormones (Kasson et al., 1986; Kimura et al., 1987). It also inhibits feeding behaviour and regulates fat and protein metabolism (Sekar and Chow, 2013). Tetrapod SCTR belongs to the PAC_1/VPAC subfamily group (a.k.a. secretin-GPCRs or E-group) that shared the same ancestral gene as the vertebrate glucagon and parathyroid receptors and emerged after the protostome-deuterostome divergence (Harmar, 2001; Cardoso et al., 2004, 2005, 2006; Schioth and Fredriksson, 2005; Hwang et al., 2013;

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Mirabeau and Joly, 2013; Cardoso et al., 2014). In vertebrates' six functional PAC_1/VPAC members exist and they are named after the neuropeptides that activate them. The pituitary adenylate cyclase-activating polypeptide (PACAP) receptor (PAC_1) and vasoactive intestinal peptide (VIP) receptors ($VPAC_1$ and $VPAC_2$) are stimulated by PACAP and VIP with the exception of fish $VPAC_2$ that is activated by peptide histidine isoleucine (PHI) (Wu et al., 2008). Growth hormone releasing hormone (GHRH) receptor (GHRHR) is activated by GHRH and PACAP-related peptide (PRP) stimulates the PRP receptor (PRPR) and has not so far been identified in mammals (Tam et al., 2013).

Subsequent to isolation of rat SCTR (Ishihara et al., 1991) homologues were isolated in human (Chow, 1995; Jiang and Ulrich, 1995; Patel et al., 1995), mouse (Vassilatis et al., 2003), cattle (Meuth-Metzinger et al., 2005) and rabbit (Svoboda et al., 1998). Recently, SCTR was isolated in chicken, amphibian (*Xenopus laevis* and *Rana rugulosa*) and African lungfish (*Protopterus dolloi*) (Tam et al., 2011; Wang et al., 2012). Characterization of the non-mammalian receptors revealed that in common with mammals, SCT stimulates a rise in intracellular cAMP and increases mobilization of intracellular calcium (iCa^{2+}) (Tam et al., 2011; Wang et al., 2012) and the chicken and amphibian peptide homologues share a conserved role and stimulate pancreatic secretion (Dockray, 1975a, 1975b; Tam et al., 2011).

SCTR is the most recently identified member of the vertebrate neuropeptide Class 2 B1 (GPCR) family in teleosts (Wang et al., 2012; Hwang et al., 2013) from which both receptor and SCT genes were thought to be absent (Cardoso et al., 2005, 2006, 2007a; Roch et al., 2009; Cardoso et al., 2010; Tam et al., 2011). Recently, using different strategies and combining gene linkage synteny analysis allied to DNA amplification techniques the first teleost SCTR cDNA was isolated from the zebrafish brain (Wang et al., 2012). Intriguingly so far no gene encoding the isolated receptor has been identified in the zebrafish genome assembly (Wang et al., 2012) however SCTR genes have been identified in other teleost genomes (Hwang et al., 2013).

Teleost fishes comprise the largest and most speciose group of vertebrates on earth. Their evolution was affected by a specific whole genome duplication (teleost specific gene duplication, TSGD or 3R) that occurred early in the *Teleostei* radiation (Volff, 2005; Ravi and Venkatesh, 2008). Fish genomes have a higher rate of chromosomal rearrangements, gene-linkage disruptions and contain faster evolving protein-coding sequences compared with mammals (Ravi and Venkatesh, 2008; Lu et al., 2012). This rapid evolution has been shown to affect the existence and persistence of other Class 2 B1 GPCRs in the genome and the evolutionary context of the fish SCTR remains to be established (Cardoso et al., 2004, 2005; Fradinger et al., 2005; Cardoso et al., 2007b; Roch et al., 2009; Irwin and Prentice, 2011; Hwang et al., 2013). The present study will integrate and extend the model previously developed for PAC_1/VPAC subfamily group evolution by integrating and extending knowledge about SCTR and SCT in the fishes in relation to the chordates and terrestrial vertebrates.

2. Methods

2.1. Sequence database searches and data retrieval

SCTR genes were procured in the lamprey (jawless fish), cartilaginous fish (holocephalan), ray-finned fishes and in lobe-finned fish genomes publicly available. Nine genomes from ray-finned fishes were explored which included two pufferfish (*Tetraodon nigroviridis*; *Takifugu rubripes*); stickleback (*Gasterosteus aculeatus*); nile tilapia (*Oreochromis niloticus*); medaka (*Oryzias latipes*); platyfish (*Xiphophorus maculatus*); Atlantic cod (*Gadus morhua*); cavefish (*Astyanax mexicanus*) and the primitive freshwater ray-finned fish the spotted gar (*Lepisosteus oculatus*); the genomes were available from the ENSEMBL (http://www.ensembl.org, accessed October 2013) database. The lobe-finned fish coelacanth (*Latimeria chalumnae*) genome assembly was accessed from the ENSEMBL (http://www.ensembl.org, accessed October 2013) database. The bait was the deduced amino acid sequence of the zebrafish (*Danio rerio*) SCTR homologue (ACC86056.1, (Wang et al., 2012)) of the human receptor. Searches for SCTR transcripts were also performed in teleost fish (taxid:32443) EST databases available from NCBI (http://www.ncbi.nlm.nih.gov/, accessed October 2013). Searches in the genomes of the marine lamprey (*Petromyzon marinus*, http://www.ensembl.org, accessed October 2013) and the Japanese lamprey (*Lethenteron japonicum*, http://jlampreygenome.imcb.a-star.edu.sg, November 2013) and in the cartilaginous fish, the elephant shark (*Callorhinchus milii*, http://esharkgenome.imcb.a-star.edu.sg, November 2013) were also carried out using the zebrafish SCTR, the hagfish (*Eptatretus burger*) and lamprey VPACs (Ng et al., 2012).

The genomes of terrestrial vertebrates, the amphibian (*Xenopus tropicalis*), Anole lizard (*Anolis carolinensis*) and chicken (*Gallus gallus*) were also mined for the receptor gene homologues. The existence of SCTR in early chordate genomes, the acorn worm (*Saccoglossus kowalevskii*, https://www.hgsc.bcm.edu/), sea urchin (*Strongylocentrotus purpuratus*, http://metazoa.ensembl.org/), amphioxus (*Branchiostoma floridae*, http://genome.jgi-psf.org/Brafl1/Brafl1.home.html) and Ciona (*Ciona intestinalis*, http://www.ensembl.org/) were also explored. Searches for other members of the PAC_1/VPAC subfamily (PAC_1, $VPAC_1$, $VPAC_2$, GHRHR and PRPR) were also performed in the above-mentioned genomes.

The existence of a putative SCT gene in genome assemblies was determined using the databases described above and also teleost EST libraries that were searched with the *X. laevis* (NP_001267540.1), chicken (NP_001020004.1) and human (NP_068739.1) mature peptide transcripts.

2.2. Phylogenetic analysis

Phylogenetic analysis of SCTR was performed using the deduced amino acid sequence of the seven TM domains and intra and extracellular loops (Supplementary File 1). Eighty-three sequences, from 19 different vertebrates including 14 fish were used and contained representatives of the SCT, PACAP, VIP, GHRH and PRP receptors and of the GCG and PTH receptor families. The receptor sequence alignment was submitted to ProtTest (2.4) to select the best model to study protein evolution according to the Akaike Information Criterion (AIC) statistical model (Abascal et al., 2005). Bootstrapping analysis was used to assign measures of accuracy of the phylogenetic clades (Felsenstein, 1985). ML analysis was constructed in the PhyML program (v3.0 aLRT) with receptor sequences that were aligned using ClustalW (2.0.3) (Thompson et al., 1997). Data was also analysed using the NJ method (Saitou and Nei, 1987) implemented in the Mega 5.2 program (Tamura et al., 2011). The phylogenetic trees were built using the JTT substitution model (Jones et al., 1992). ML analysis included fixed proportion of invariant sites (0.02), 4 gamma-distributed rate categories to account for rate heterogeneity across sites and gamma shape parameter was fixed (1.03). Reliability for internal branching was assessed using 100 bootstrap replicates. NJ analysis was performed with 4 gamma-distributed rate categories, fixed gamma parameter (1.03) and reliability of internal branches assessed using 1000 bootstrap replicates. ML and NJ trees were rooted with the vertebrate PTHR subfamily cluster that contained the receptors from human (ENSP00000321999), chicken (ENSGALP00000008782), zebrafish (ENSDARP00000091807 and ENSDARP00000027674) and spotted-gar (ENSLOCP00000011589 and ENSLOCP00000014456).