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Peroxisome proliferator-activated receptor gamma (PPARγ) in yellow catfish *Pelteobagrus fulvidraco*: Molecular characterization, mRNA expression and transcriptional regulation by insulin *in vivo* and *in vitro*

Jia-Lang Zheng [a,b,c,1], Mei-Qin Zhuo [b,c,1], Zhi Luo [b,c,*], Ya-Xiong Pan [b,c], Yu-Feng Song [b,c], Chao Huang [b,c], Qing-Ling Zhu [b,c], Wei Hu [b,c], Qi-Liang Chen [b,c]

[a] National Engineering Research Center of Marine Facilities Aquaculture, Zhejiang Ocean University, Zhoushan 316022, China
[b] Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture of P.R.C., Fishery College, Huazhong Agricultural University, Wuhan 430070, China
[c] Freshwater Aquaculture Collaborative Innovative Centre of Hubei Province, Wuhan 430070, China

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ABSTRACT

Peroxisome proliferator-activated receptor gamma (PPARγ) is ligand-inducible transcription factor and has important roles in lipid metabolism, cell proliferation and inflammation. In the present study, yellow catfish *Pelteobagrus fulvidraco* PPARγ cDNA was isolated from liver by RT-PCR and RACE, and its molecular characterization and transcriptional regulation by insulin *in vivo* and *in vitro* were determined. The generation of PPARγ1 and PPARγ2 was due to alternative promoter of PPARγ gene. PPARγ1 and PPARγ2 mRNA covered 2426 bp and 2537 bp, respectively, with an open reading frame (ORF) of 1584 bp encoding 527 amino acid residues. Yellow catfish PPARγ gene was organized in a manner similar to that of their mammalian homologs, implying a modular organization of the protein's domains. A comparison between the yellow catfish PPARγ amino acid sequence and the correspondent sequences of several other species revealed the identity of 55–76.2%. Two PPARγ transcripts (PPARγ1 and PPARγ2) mRNAs were expressed in a wide range of tissues, but the abundance of each PPARγ mRNA showed the tissue- and developmental stage-dependent expression patterns. Intraperitoneal injection of insulin *in vivo* significantly stimulated the mRNA expression of total PPARγ and PPARγ1, but not PPARγ2 in the liver of yellow catfish. In contrast, incubation of hepatocytes with insulin *in vitro* increased the mRNA levels of PPARγ1, PPARγ2 and total PPARγ. To our knowledge, for the first time, the present study provides evidence that PPARγ1 and PPARγ2 are differentially expressed with and among tissues during different developmental stages and also regulated by insulin both *in vivo* and *in vitro*, which serves to increase our understanding on PPARγ physiological function in fish.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-inducible transcription factors belonging to the nuclear hormone receptor superfamily. To date, three PPAR isotypes-α, β and γ, encoded by separate genes and showing different tissue distribution patterns have been identified (Desvergne and Wahli, 1999; Escher et al., 2001). Among three PPAR isotypes, PPARγ is highly expressed in adipose tissue and has important roles in lipid metabolism, cell proliferation and inflammation (Tsai et al., 2008). In mammals, two transcripts of PPARγ with different lengths of the N-terminal, γ1 and γ2, have been found in mice (Tontonoz et al., 1994b; Zieleniak et al., 2008). The expression of these two transcripts results from differential promoter use and alternative RNA splicing (Zhu et al., 1995). The PPARγ2 transcript was predominantly expressed in adipose tissue and had a key regulatory role in the induction and maintenance of the adipocyte phenotype (Tontonoz et al., 1994a; Fajas et al., 1997), whereas PPARγ1 was relatively widely expressed (Fajas et al., 1997; Mukherjee et al., 1997).

Abbreviations: aa, amino acid; DBD, DNA-binding domain; LBD, ligand-binding domain; NJ, neighbor-joining; nt, nucleotide; ORF, open reading frame; PPAR, peroxisome proliferator-activated receptor; RACE, rapid amplification of cDNA ends; SEM, standard errors of means; UTR, untranslated regions.

* Corresponding author at: Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture of P.R.C., Fishery College, Huazhong Agricultural University, Wuhan 430070, China. Fax: +86 27 8728 2114.

E-mail addresses: luozhi99@mail.hzau.edu.cn, luozhi99@aliyun.com (Z. Luo).

[1] These authors equally contributed to the work.

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At present, PPARγ has been identified and cloned in many fish species. For example, a single PPARγ transcript has been identified in *Rachycentron canadum* (Tsai et al., 2008), *Paralichthys olivaceus* (Cho et al., 2009), *Dicentrarchus labrax* (Boukouvala et al., 2004), *Salmo trutta* (Batista-Pinto et al., 2005), *Pleuronectes platessa* and *Sparus aurata* (Leaver et al., 2005), *Takifugu rubripes* (Kondo et al., 2007), *Danio rerio* (Ibabe et al., 2005) and *Chelon labrosus* (Raingeard et al., 2009). However, the exact number of genes and/or the presence of distinct PPARγ have not been determined in fish. In *Salmo salar*, two PPARγ transcripts that differed in the length due to alternative usage of the multiple polyadenylation were sequenced and Northern blot analysis revealed a third PPARγ transcript that encoded a C-terminally truncated variant (Andersen et al., 2000). A short PPARγ transcript was also found in *S. salar*, which represented an alternatively spliced form of PPARγ that lacked the first 102 nucleotides of exon 3 (Todorcevic et al., 2008). The further study suggested that PPARγ short was induced during adipocyte differentiation, indicating that this transcript played a role in lipid accumulation in adipocytes, whereas the PPARγ long was induced in the early phase of cultivation and repressed at later stages of differentiation. Studies also suggested that the fish PPARγ gene was not activated by common mammalian PPARγ-specific ligands (Leaver et al., 2005; Kondo et al., 2007), indicating a marked difference in structure and function of PPARγ gene between fish and mammals.

Insulin is a peptide hormone that stimulates cell growth and differentiation, and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown (Saltiel and Kahn, 2001). Insulin resistance is a widely pathological disease in humans (Reaven, 1988). Some PPARγ activators such as thiazolidinediones (TZD) are widely reported to improve insulin sensitivity (Benton et al., 2010), suggesting that the regulation of insulin on metabolism could be mediated by PPARγ. However, information on the direct effect of insulin on PPARγ expression is very scarce. Limited studies pointed out that insulin up-regulated both PPARγ1 and PPARγ2 expressions in isolated human adipocytes (Vidal-Puig et al., 1997), and no information was available in fish.

Yellow catfish *Pelteobagrus fulvidraco* is an omnivorous, freshwater species of fish with increasing interest in Chinese inland aquaculture. As a result of the rapid expansion of intensive aquaculture for yellow catfish, excess lipid deposition in the adipose tissue and liver of the fish species has seriously impacted growth performance and health. Recently, we cloned the partial cDNA sequence of PPARγ and investigated mRNA tissue expression profiles of the single PPARγ gene (Zheng et al., 2013). As a continuation of our studies involved in the structure and functions of the gene, the present study cloned the full-length cDNA sequences of two PPARγ transcripts, and determined their tissue-specific and developmental expression profiles. Meanwhile, the patterns of two PPARγ transcripts mRNA expression under insulin treatment *in vivo* and *in vitro* were evaluated in this fish species. The present study would extend our understanding on the physiological function of PPARγ gene in fish.

2. Materials and methods

Here, two experiments were conducted. The first experiment was involved in the PPARγ cDNA cloning, mRNA expression patterns of various tissues and during different developmental stages. The second experiment was designed to evaluate the regulation of PPARγ by insulin *in vivo* and *in vitro*. We assured that the experiments performed on animals and cells followed the ethical guidelines of Huazhong Agricultural University.

2.1. Experiment 1: PPARγ cDNA cloning and mRNA expression of various tissues and during different developmental stages

2.1.1. Fish culture and sampling

Two groups of yellow catfish were used. The first group of three fish (mean weight: 25 g) was obtained from a local commercial farm. Liver, heart, white muscle, intestine, brain, visceral adipose, spleen, kidney and gill were collected, frozen immediately in liquid nitrogen and stored at −80 °C until their use in tissue mRNA expression analysis.

The second group of yellow catfish came from the same farm and was used in the developmental study. Three different developmental stages were chosen: larvae (body weight 0.15 ± 0.03 g, 1 month old), juvenile (body weight 4.5 ± 0.5 g, 2 month old), and adult (body weight 20.6 ± 1.8 g, 4 month old). The larval yellow catfish were hatched in June 2013, raised in indoor tanks. They were provided with commercial Haid® diets to satiation twice a day and continuous aeration to maintain the dissolved oxygen level near saturation. Dechlorinated tap water in each tank was replenished 100% twice daily, before feeding. Care was taken to ensure that no uneaten feed remained in the tanks during feeding. Fecal matter was also quickly removed during the experiment. The experiment was conducted at ambient temperature and subjected to natural photoperiod (approximately 14 h light/10 h dark). Water quality parameters were monitored twice a week in the morning. Water temperature ranged from 28 °C to 33 °C; dissolved oxygen 5.8 mg l^{-1}; total ammonia–nitrogen 0.042–0.051 mg l^{-1}.

At the end of the first, second and fourth month, fish were starved for 24 h before sampling. Fish were euthanized (MS-222 at 10 mg l^{-1}). Liver, heart, white muscle, intestine, brain, visceral adipose, spleen, kidney and gill were removed on the ice, rapidly frozen in liquid nitrogen for subsequent analysis. All reagents used for cloning and expression analysis were purchased from TaKaRa (Tokoyo, Japan) unless otherwise stated. The sequencing was also undertaken in TaKaRa.

2.1.2. RNA isolation, reverse transcription and fragment amplification

Frozen tissues were powdered in a liquid nitrogen-chilled mortar and pestle. Total RNA was extracted from liver, heart, white muscle, intestine, brain, visceral adipose, spleen, kidney and gill using TaKaRa RNAiso plus based on the acid guanidinium thiocyanate–phenol–chloroform extraction method. Contaminating genomic DNA was eliminated using RNase-free Dnase, according to the manufacturer's instructions. Two microgram of total RNA was used for reverse transcription with TaKaRa PrimeScript™ RT-PCR Kit, following the protocol of the manufacturer. Two microliters of the cDNA obtained were used as template for PCR. Amplifications by PCR were carried out (30 amplification cycles at 95 °C for 1 min, 57 °C for 1 min and 72 °C for 1 min) using oligonucleotide primers previously deduced from alignment (using Clustal-w software, Infobiogen) of corresponding sequences available in GenBank database from different fish and mammalian species (primers shown in Supplementary Table 1). The resulting target fragments were purified using the Agarose Gel DNA Fragment Recovery Kit Ver.2.0, subcloned using the PMD19-T Easy Vector System. The plasmid was used for transformation of JM109 Competent Cells and six clones with inserts were sequenced.

2.1.3. Full-length PPARγ cDNA amplification

Full-length PPARγ cDNA of yellow catfish was obtained by the procedures of rapid amplification of cDNA ends (RACE) method with the gene-specific primers designed based on the partial sequence. The primers used for RACE are shown in Supplementary Table 1. 3′ RACE was performed using 3′-Full RACE Core Set, according to the manufacturer's instructions. Firstly, reverse transcription of 2 μg of total RNA was performed with Oligo(dT)16AP