Comparative Biochemistry and Physiology, Part B 204 (2017) 69–76

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part B

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

Two grass carp (*Ctenopharyngodon idella*) insulin-like growth factor-binding protein 5 genes exhibit different yet conserved functions in development and growth

Guo-Dong Zheng [1], Chun-Xue Zhou [1], Si-Tong Lin, Jie Chen, Xia-Yun Jiang, Shu-Ming Zou *

Key Laboratory of Genetic Resources for Freshwater Aquaculture and Fisheries, Shanghai Ocean University, 999 Huchenghuan Road, Shanghai 201306, China

ARTICLE INFO

Article history:
Received 3 July 2016
Received in revised form 23 November 2016
Accepted 24 November 2016
Available online 30 November 2016

Keywords:
Ctenopharyngodon idella
igfbp5
Growth hormone
Fasting
Expression analysis

ABSTRACT

Insulin-like growth factor binding-protein 5 (*igfbp5*), the most conserved member of the IGFBP family in vertebrates, plays a critical role in controlling cell survival, growth, differentiation, and apoptosis. Here, we characterized the expression patterns of *igfbp5a* and *igfbp5b* in grass carp (*Ctenopharyngodon idella*), which are retained in many fish species, likely from the teleost-specific whole-genome duplication. Both *igfbp5a* and *igfbp5b* encode 268- and 263-aa peptides, respectively, which share a sequence identity of 71%. Their mRNAs are not detected in zygotes. At 14 hpf, grass carp *igfbp5b* mRNA was detected in the somites, while *igfbp5a* mRNA has some possible signal around the eye and head region. At 24 hpf, both *igfbp5a* and *igfbp5b* mRNA appear to be limited to the presomitic mesoderm. At 36 hpf, *igfbp5a* mRNA was only detected in the midbrain, while *igfbp5b* mRNA was detected in both the midbrain and notochord. Overall, both mRNAs were expressed in most adult tissues. *igfbp5a* and *igfbp5b* were significantly upregulated in the muscle and liver after injection of 10 μg per kilogram body weight of zebrafish growth hormone (zGH), while their hepatic expression was downregulated by 50 μg zGH. During fasting, both *igfbp5a* and *igfbp5b* mRNAs were significantly downregulated in the muscle but upregulated in the liver. Collectively, the results suggest that the two *igfbp5* genes play important but different roles in the regulation of growth and development in grass carp.

1. Introduction

The bioactivities of insulin-like growth factors (IGFs) are modulated by a group of specific high-affinity IGF-binding proteins (IGFBPs). The peptides of IGFBPs constitute a separate protein family, and 6 identified members, designated as *igfbp1* to *igfbp6*, have been shown to be ancestral to jawed vertebrates (Drop, 1991; Shimasaki et al., 1991a, 1991b; Firth and Baxter, 2002; Macqueen et al., 2013). *igfbp5* can modulate IGF activity by binding to it and in turn regulating its binding to IGF-1 receptors on the cell surface (Jones and Clemmons, 1995; Zapf, 1995). This peptide is the most conserved member of the IGFBP family in all vertebrates (Duan, 1997; Cobb et al., 2004) and plays a critical role in controlling cell survival, growth, differentiation, and apoptosis (Flint et al., 2000). Mammal *igfbp5* has also been shown to potentiate IGF actions via its interactions with extracellular matrix components (Mohan et al., 1995; Parker et al., 1998; Ren et al., 2008). Some studies suggest that *igfbp5* itself acts as a growth factor with cellular effects that are not dependent on its IGF-binding ability (Abrass et al., 1997; Berfield et al., 2000; Miyakoshi et al., 2001; Hsieh et al., 2003).

Mammals have single copy of the *igfbp5* gene. In teleost fish, duplicated copies of *igfbp5* genes are retained, presumably due to a whole genome duplication event in the teleost ancestor (Macqueen et al., 2013). Recent studies demonstrate that the *igfbp5* gene is duplicated in the zebrafish genome: *igfbp5a* is present on chromosome 6 and *igfbp5b* is present on chromosome 9 (Dai et al., 2010). The duplicated zebrafish *igfbp5s* have the same domain arrangement, high sequence identity, and the ability to bind IGFs, but have evolved divergent regulatory mechanisms and distinct biological properties by partitioning of ancestral structural domains (Dai et al., 2010). Duplicate *igfbp5s* are also found in Atlantic salmon that are 1-to-1 orthologues of the zebrafish duplicates (Bower et al., 2008; Macqueen et al., 2013). Moreover, duplicated *igfbp5b* genes (*igfbp5b1* and *-5b2*) are retained in salmonid species from a later, salmonid-specific whole genome duplication event (Macqueen et al., 2013). Although duplicate *igfbp5* genes have been identified in several teleost fish, molecular information of duplicated *igfbp5* genes is still limited in aquaculture fish.

It has been reported that the postnatal growth-promoting effects of GH may be mediated mainly by IGF1 (Duan, 1997; Le Roith et al., 2001), and mammalian *igfbp5* expression corresponds with the activity of IGFs during development (Cheng et al., 1996; Kalus et al., 1998; Nguyen et

* Corresponding author at: Key Laboratory of Freshwater Aquatic Genetic Resources, Shanghai Ocean University, 999 Huchenghuan Road, Shanghai 201306, China.
E-mail address: smzou@shou.edu.cn (S.-M. Zou).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cbpb.2016.11.008
1096-4959/

al., 2013). The *igfbp5* mRNA can be regulated by GH injection in Mozambique tilapia (Breves et al., 2014), and the expression patterns of GH were affected by nutritional status in rabbitfish and channel catfish (Small and Peterson, 2005; Ayson et al., 2007). The regulation of *igfbp5* genes during in vitro myogenesis and nutritional/hormonal manipulation has been shown in Atlantic salmon (Bower et al., 2008; Bower and Johnston, 2010; Macqueen et al., 2013). Furthermore, a dynamic nutritional and time-dependent response in the expression of *igfbp5* was described in the skeletal muscle of flounder (Safian et al., 2012). In rainbow trout, *igfbp5* mRNA was found to be significantly upregulated during the post-vitellogenesis stage (Kamangar et al., 2006) and the expression of duplicated *igfbp5* genes (both salmonid-specific duplicates and teleost duplicates) was demonstrated in response to manipulation of immune status and during development (Alzaid et al., 2016). Thus, the regulatory mechanism of *igfbp5* seems related to energy metabolism and growth. However, no information shows duplicated *igfbp5*s expression in grass carp may be regulated by GH or feeding/fasting treatment.

Grass carp is the principal species in Chinese freshwater fish polyculture systems, and its total production exceeds 5 million tons in 2013 (FBMA, 2014). Fish, including grass carp, tend to grow throughout life to accumulate muscle tissue (Mommsen, 2001). A brood of the herbivorous grass carp takes at least 5 years to attain sexual maturity and its rapid growth before maturity provides a useful model for our study (Xia et al., 2010; Jiang et al., 2011; Yuan et al., 2011). In the present study, duplicated *igfbp5* cDNAs were cloned and identified from grass carp, the sequence and expression patterns of both *igfbp5* genes were examined in adult tissues and at different embryo stages, and their responses to growth hormone and fasting treatment were studied.

2. Materials and methods

2.1. Ethics statement

All experiments were permitted by the Shanghai Ocean University and conducted following the guidelines approved by the Shanghai Ocean University Committee on the Use and Care of Animals.

2.2. Experimental fish

Grass carp were obtained from the Bream Genetics and Breeding Center of Shanghai Ocean University, Shanghai, China. Embryos were generated by artificial insemination of grass carp (*C. idella*). Egg fertilization and embryonic development were carried out at room temperature (approximately 22 °C). Fertilized eggs (100 − 200) were placed in a Petri dish (15-cm diameter). Water in the Petri dish was replaced every 4 h with well-aerated water to maintain normal dissolved oxygen (DO) values at 7.0 ± 0.5 mg/L during embryogenesis. To prevent pigmentation, embryos collected at 14, 24, 36 hour post-fertilization (hpf) for in situ hybridization analysis were manually dechorionated and fixed overnight at 4 °C in 4% phosphate-buffered paraformaldehyde and at − 20 °C in methyl alcohol. Five 3-year old adult (unmaturity adult) grass carp (~8.0 kg) were euthanized by immersion in 100 mg/L MS-222 (Sigma, St. Louis, MO, USA) for 5 min. Tissues including brain, heart, liver, spleen, kidney, gut, eyes, skin, muscle, gill, gill filament, and fin were rapidly dissected, frozen in liquid nitrogen and stored at − 80 °C till use.

2.3. Fasting and zGH treatments

For fasting treatment, 1-year old juvenile grass carp (~20 g each) were stocked (30 fish per tank) in two 150 L indoor continuous flow system tanks. A total of six fish (3 per tank) were collected at days 2, 4 and 6 during fasting treatment and at days 3 and 6 during refeeding treatment. Five fish in a feeding control tank were taken at the same times. The GH treatments were performed according to (Lin et al., 2015), 18 juveniles were cultured in three 150-L indoor continuous flow system tanks. After 3 days without feeding, six fish in each of three tanks were anesthetized in 100 mg/L MS-222 and then given an intraperitoneal injection of phosphate-buffered saline (PBS, control) or 10 or 50 μg of recombinant zebrafish GH (zGH, Shanghai Gaochuang Chemical Company, China) per gram body weight in a volume of 100 μL. After 12 h, six fish were sampled for each experimental treatment. The tissues were rapidly excised, frozen in liquid nitrogen, and stored at − 80 °C until used.

2.4. Molecular cloning of igfbp5a and -5b cDNAs in grass carp

Total RNA was isolated from grass carp embryos at 32 hpf using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and subsequently treated with DNase (Promega, Madison, WI, USA) to remove contaminating genomic DNA. First-strand cDNA was reverse-transcribed from total RNA using Reverse Transcriptase M-MLV (Takara, Tokyo, Japan) with oligo-dT primers according to the manufacturer's instructions. To clone grass carp *igfbp5a* and *-5b* partial cDNA fragments, gene-specific primers *igfbp5a*-F/-R (*igfbp5a*) and *igfbp5b*-F/-R (*igfbp5b*) were designed based on the zebrafish (*Danio rerio*) *igfbp5a* and *igfbp5b* mRNA sequences (GenBank Accession No. GQ892882.1, 1817 bp; AY100478.1, 4330 bp) (Table 1). Reverse transcription (RT) PCR was performed to amplify partial cDNA fragments of grass carp *igfbp5a* using the primer pair *igfbp5a*-F/-R (Table 1). A 752-bp partial PCR fragment of grass carp *igfbp5a* was cloned, sequenced and used to design nested gene-specific primer pair 5′-*igfbp5a* race/nest and 3′-*igfbp5a* race/nest (Table 1) for 5′ RACE and 3′ RACE analysis, respectively. RT-PCR was performed to amplify partial cDNA fragments of grass carp *igfbp5b* using the primer pairs *igfbp5b*-F/-R (Table 1). A 550-bp partial PCR fragment of *igfbp5b* was cloned, sequenced and used to design nested gene-specific primer pairs 5′-*igfbp5b* race/nest and 3′-*igfbp5b* race/nest (Table 1) for 5′ RACE and 3′ RACE analysis, respectively. The 5′ and 3′ ends of the *igfbp5a* and *-5b* mRNAs were amplified using a SMART RACE cDNA amplification kit (Clontech, Mountain View, CA, USA), following the manufacturer's protocol. PCR products were gel-purified, ligated into the T/A cloning vector pMD-19 T (Takara, Japan) and transformed into *Escherichia coli DH5α* competent cells. Positive clones were examined by PCR and direct Sanger sequencing.

Table 1
Primer sequences used in this study.

Primers name	Primer sequence (5′-3′)	Assay technique
igfbp5a F	GCGTGCGGCGTTTAACACCGGGAC	Fragment PCR
igfbp5a R	TTGTCTACACACCAGCAGAT	
igfbp5b F	GAAGGCGCTCTCCATGTGTCCT	Fragment PCR
igfbp5b R	GTGGGCCCTAGGTCTGGTGATC	
3′-*igfbp5a* race	ATCGGAATGGACAAACATCAGTC	*igfbp5a* 3′ RACE
3′-*igfbp5a* nest	TCATGGCTCTTTCTCTGTACCTTC	
3′-*igfbp5b* race	CACGCGTCATGGCTCTTTCCCTC	*igfbp5b* 3′ RACE
3′-*igfbp5b* nest	ATCTGCTGGTGCGTGGACAAATAT	
5′-*igfbp5a* race	TGGTCCCGTTGGAATAATGGAACC	*igfbp5a* 5′ RACE
5′-*igfbp5a* nest	TCGGCATATGAAGTGGTTTGTATA	
5′-*igfbp5b* race	TTAAGGGGAATTTTAGGACTCTGG	*igfbp5b* 5′ RACE
5′-*igfbp5b* nest	TACACACTCCCCTGCCGTGCAACA	
igfbp5a qRT F	AGCAACGGCAACAACAACGA	qRT-PCR
igfbp5a qRT R	GATCCCACCAACCCATGAAGA	
igfbp5b qRT F	AAGGGCTTCTTCAAGCGCAA	qRT-PCR
igfbp5b qRT R	TGGTGATCAGGGTGGGTTGA	
β-actin qRT F	TGCCATGTATGTGGCCATCC	qRT-PCR
β-actin qRT R	TCTTTCGGCTGTGGTGGTGA	
igfbp5a in situ F	CTTCCCCCGAATTCCATTGATTCC	WISH
igfbp5a in situ R	TGACCCCTTGTCCTGAGTTTAGCC	
igfbp5b in situ F	GTGCGCAAGGACAAGAAGAAACAA	WISH
igfbp5b in situ R	TCCCCAATGAATAAGTGCTCTGAC	