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Molecular characterization and expression profiles of insulin-like growth factors in yellowtail kingfish (*Seriola lalandi*) during embryonic development

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Abstract In this study, to understand the role of the insulin-like growth factor (IGF) system in the regulation of early development in yellowtail kingfish (YTK, *Seriola lalandi*), an economically important marine fish species with a high potential for aquaculture, we first cloned the full-length cDNAs for *igf1* and *igf2* from the liver. YTK *igf1* cDNA was 1946 base pairs (bp) in length with an open reading frame (ORF) of 558 bp encoding preproIGF1 of 185 amino acids (aa). The preproIGF1 consisted of 44 aa for the signal peptide, 68 aa for the mature peptide comprising B, C, A, and D domains, and 73 aa for the E domain. YTK *igf2* cDNA had an ORF of 648 bp that encoded a total of 215 aa spanning the signal peptide (47 aa), the mature peptide (70 aa), and the E domain (98 aa). At the protein level, both YTK IGF1 and IGF2 exhibited high sequence identities with their corresponding fish counterparts, respectively. Subsequently, quantitative RT-PCR analysis indicated that the highest level of *igf1* mRNA expression was recorded in the gonad and liver, while the *igf2* mRNA expression was most abundant in the gill and liver. In addition, both *igf1* and *igf2* were detected in all stages of embryonic development and exhibited different gene expression patterns, supporting that IGF1 and IGF2 could be functional and play important roles during YTK embryogenesis. Overall, this initial study of IGF1 and IGF2 provides an insight into the endocrine mechanism involved in the early development of yellowtail kingfish.

Keywords Insulin-like growth factor · cDNA cloning · Embryonic development · *Seriola lalandi*

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Introduction

The insulin-like growth factors (IGFs) are evolutionarily ancient growth factors and play a critical role in the control of growth, development, and reproduction in vertebrates. IGFs are single-chain polypeptides which are structurally related to proinsulin. PreproIGF consists of a signal peptide, B, C, A, D, and E domains in turn from the N-terminal, while preproinsulin consists only a signal peptide, B, C, and A domains (Caruso and Sheridan 2011; Moriyama et al. 2000; Wood et al. 2005). The signal peptide and E domain are proteolytically removed from the preprohormones to produce mature IGFs composed of 67–70 amino acids, depending on the species. Three disulfide bonds are formed in IGFs

during the processing: two are between B and A domains and one is in A domain (Moriyama et al. 2000; Wood et al. 2005). Both IGF1 and IGF2 are present in various vertebrates, and their structures are highly conserved. Recently, the potential presence of a novel IGF form (called IGF3) encoded by a separate gene has been identified in some bony fish species, such as tilapia, zebrafish, orange-spotted grouper, and common carp (Song et al. 2016; Wang et al. 2008; Yang et al. 2015).

Regulation of growth is very important to aquaculture production and the growth hormone (GH)-IGF axis plays a major role in the control of growth. IGFs are the primary mediators of the growth-promoting effects of GH in fish and other vertebrates and have been shown to operate in an autocrine, paracrine, and endocrine manner (Reindl and Sheridan 2012; Wood et al. 2005). Administration of GH was shown to increase hepatic *igf1* and *igf2* mRNAs in rainbow trout (Shamblott et al. 1995; Very et al. 2008), common carp (Tse et al. 2002; Vong et al. 2003), coho salmon (Pierce et al. 2010, 2004), redbanded seabream (Ponce et al. 2008), pejerrey (Sciara et al. 2008), tilapia (Eppler et al. 2010; Pierce et al. 2011; Shved et al. 2011), orange-spotted grouper (Wang et al. 2016), and white seabream (Perez et al. 2016). Furthermore, multiple lines of evidence demonstrated that extrahepatic *igf1* and *igf2* mRNAs were also positively regulated by GH in various species of fish (Eppler et al. 2010; Perez et al. 2016; Shamblott et al. 1995; Shved et al. 2011; Tse et al. 2002; Vong et al. 2003; Yang et al. 2015). On the other hand, IGF may directly act on pituitary to modulate GH synthesis and secretion by negative feedback. For instance, both IGF1 and IGF2 were equally potent in inhibiting GH release from dispersed pituitary cells of turbot (Duval et al. 2002).

It should be noted that IGF peptides in fish have been associated not only with growth, but also with metabolism, reproduction, osmoregulation, and development (Reinecke 2010a, b; Reinecke et al. 2005; Wood et al. 2005). Especially, the expression and cellular localization of IGF during early development have been extensively investigated in tilapia (Berishvili et al. 2010, 2006a, b; Wang et al. 2008) and gilthead seabream (Perrot et al. 1999; Radaelli et al. 2003), suggesting a crucial role of IGF in developing teleosts by autocrine/paracrine means of regulation. Because fish embryos develop externally, they provide excellent animal models for understanding the regulatory roles of IGF in vertebrate embryonic development (Duan 1998). It has been shown that all *igf1*, *igf2*, and *igf3* genes exhibited distinct and dynamic expression profiles during zebrafish embryogenesis (Li et al. 2014). In addition, developmental changes in transcripts of *igf1* and *igf2* were also monitored in rainbow trout (Li et al. 2007; Malkuch et al. 2008), gilthead seabream (Perrot et al. 1999), rabbitfish (Ayson et al. 2002), hybrid (channel × blue) and channel catfish (Peterson et al. 2005). Notably, the expression pattern of the two *igf* genes during embryogenesis is species- and gene-dependent. Taken together, the presence of the components of the IGF system in fish embryos may indicate that these growth factors are important during embryonic development of fish, and differences in the expression levels of the IGF forms could implicate differential regulation of gene expression and different roles in teleosts.

Yellowtail kingfish (YTK, *Seriola lalandi*) is one of the larger members of the genus *Seriola* which includes highly active pelagic fish belonging to the Carangidae family, and its importance for the aquaculture industry is growing worldwide due to its fast growth, high flesh quality, and suitability for farming in both cage and recirculating aquaculture systems (Orellana et al. 2014; Sanchis-Benlloch et al. 2017). YTK is a gonochoristic species with an asynchronous oocyte development, which provides the capacity for multiple spawning within a reproductive season and its age at sexual maturation varies between sexes, between geographical locations, and within populations (Poortenaar et al. 2001; Sanchis-Benlloch et al. 2017). Failure of female broodstock to undergo final oocyte maturation, ovulation, and spawning in captivity is a common reproductive dysfunction of cultured fishes (Mylonas et al. 2004), and aquaculture of YTK largely relies on capture of wild juveniles. Despite the reproductive behavior and morphological development of embryo have been recently described in YTK (Moran et al. 2007; Yang et al. 2016), key molecular factors involved in embryogenesis such as IGFs remain to be characterized in this species. In an initial attempt to shed light on this important issue, the aims of this study were (1) to clone and sequence the full-length cDNAs for *igf1* and *igf2*, (2) to study the tissue distribution of *igf1* and *igf2* mRNAs, and (3) to examine the expression profiles of *igf1* and *igf2* during embryonic development of YTK.

Materials and methods

Molecular cloning of *igf1* and *igf2* cDNAs

All of the animal experiments were approved by the Animal Care and Use Committee of the Chinese Academy of Fishery Sciences. The fish were anesthetized with 0.05% MS222 (Sigma, Shanghai, China) and decapitated. Total RNA was extracted from the liver of YTK using the RNAiso Plus reagent (Takara, Dalian, China) according to the manufacturer's instructions. The purity and yield of RNA were assessed by a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA) and the integrity of RNA was determined on a 1% agarose electrophoresis gel with ethidium bromide staining. The first-strand cDNA was synthesized using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China) as follows: 1 μg of total RNA was incubated with gDNA Eraser at 42 °C for 2 min to eliminate contaminating genomic DNA, and then reverse transcription was performed in a volume of 20 μL of the reaction mixture containing 1 μL of PrimeScript RT Enzyme Mix I, 1 μL of RT Primer Mix, 4 μL of 5× PrimeScript Buffer, and 4 μL of RNase-free water. The reaction condition of reverse transcription was 37 °C for 15 min, followed by 85 °C for 5 s. Partial cDNA fragments were obtained by PCR using primers (Table 1) designed based on the conserved regions of IGF1 and IGF2 sequences from other teleost species already published, respectively. PCR amplification was performed with the Recombinant Taq DNA Polymerase mix (Takara, Dalian, China) and PCR conditions were as follows: denaturation at 95 °C for 3 min; followed by 35 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 30 s; and a final incubation for 5 min at 72 °C. PCR products of expected sizes were isolated, purified, and subcloned into the pEASY-T1 vector (TransGene Biotech, Beijing, China) for DNA sequencing. Based on the nucleotide sequence obtained, new primers were synthesized and 3′/5′ RACE PCRs were conducted to obtain the full-length cDNA sequences using the SMARTer® RACE Kit according to the manufacturer's protocols (Clontech, USA).

The putative signal peptide of IGF precursors was predicted using the SignalP4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). Sequence alignment of the IGF preprohormones was performed using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and was modified manually. The molecular mass and isoelectric point of mature IGFs were analyzed with ExPASy (http://web.expasy.org/compute_pi/). Phylogenetic analysis of the IGF sequences was performed using Mega6 software (Tamura et al. 2013). Sequences were aligned by ClustalW, and neighbor-joining trees were constructed using the Poisson model with 1000 bootstrap replicates.

Tissue distribution of *igf1* and *igf2* mRNAs

To detect the tissue distribution of *igf1 and igf2* mRNAs in YTK, three individuals with body weight of 283.0 ± 29.7 g and body length of 28.2 ± 0.6 cm were anesthetized with 0.05% MS222 and decapitated. Tissue samples of the brain, pituitary, eye, gill, heart, liver, spleen, head kidney, kidney, stomach, intestine, muscle, and gonad were quickly collected and immersed in RNA*later* solution (Qiagen, Germany) and then stored at − 80 °C until RNA extraction.

Embryo harvesting

Approximately 4-year-old YTK broodstock used in this study were caught off the coast of Liaoning province, China. The fish were reared in an indoor concrete tank with recirculating seawater at Dalian Fugu Fishery Co., Ltd. (Dalian, China). The fertilized eggs gathered were naturally spawned in middle April 2017, when the water temperature was 21.5 °C. Eggs were collected immediately after spawning and then incubated in 2-L beakers. The water temperature and salinity during embryo development were 21.5 °C and 31‰, respectively. The embryonic stages were classified according to the methods described in detail elsewhere (Moran et al. 2007; Yang et al. 2016) and samples of eggs at various stages were directly immersed in RNA*later* solution and then stored at − 80 °C until RNA extraction.

Real-time quantitative PCR

RNA extraction from eggs and cDNA synthesis were performed as mentioned above. Gene expression levels were determined with real-time quantitative PCR as described previously (Wang et al. 2017; Xu et al. 2017). In brief, a total of 20 μL of the PCR reaction

Table 1 Information of primers used in this study

Primer name	Primer sequence (5′-3′)	Purpose
igf1 F1	ATGTCTAGCGCTCTTTCCTTTCAG	Partial CDS
igf1 R1	CTACATTCTGTAGTTTCTGCC	
igf1 F2	AAGTGGACAAGGGCACAGAGCGC	3′ RACE PCR
igf1 R2	CCGTCGGAGTCAGGGTGAGG	5′ RACE, 1st PCR
igf1 R3	GTGAGGACACACAGCAGTAGTG	5′ RACE, 2nd PCR
igf2 F1	GCAAAGAYACGGACACCACTC	Partial CDS
igf2 R1	ATCTTCTCMGCCTGCCTCC	
igf2 F2	TTGTTTCCGTAGCTGTGACCTCAACCTG	3′ RACE, 1st PCR
igf2 F3	CAAACCCGCCAAGTCCGAAAG	3′ RACE, 2nd PCR
igf2 R2	GGTGGCCGACACGTCCCT	5′ RACE, 1st PCR
igf2 R3	CGGACTTGGCGGGTTTGGCACA	5′ RACE, 2nd PCR
UPM (long)	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	Universal primers
UPM (short)	CTAATACGAC TCACTATAGGGC	
igf1 F	TTGTGTGTGGAGAGAGAGGCTTT	qPCR
igf1 R	GAAGCAGCATTCGTCAACAATG	
igf2 F	GCAAAGATACGGACACCACTCACT	
igf2 R	CGCAGGACTGGACGAAGACAT	
18s F	TACCACATCCAAAGAAGGCA	
18s R	TCGATCCCGAGATCCAACTA	

volume contained 10 μL of 2× SYBR® *Premix Ex Taq II* (Takara, Dalian, China), 0.8 μL of forward and reverse primers (10 μM each), 2 μL of 10-fold diluted cDNA templates, and 7.2 μL of water. The amplification of samples was carried out with the Mastercycler® ep *realplex* Real-time PCR System (Eppendorf, Hamburg, Germany) using the following thermal cycling profiles: 95 °C for 30 s, and 40 cycles of 95 °C for 5 s, 58 °C for 30 s, and 72 °C for 20 s. Each cDNA sample was analyzed in triplicate. At the end of the amplification, a melting curve analysis was generated to confirm the presence of a single PCR product. PCR efficiencies ranged between 100 and 112%. The 18S gene was used as the internal reference. Relative gene expression levels were normalized to the levels of 18S and were quantified with the comparative Ct method.

Statistical analysis

Data are presented as the mean ± SEM and were analyzed by one-way ANOVA followed by Duncan's multiple range test using SPSS17.0 software (Chicago, IL, USA). Differences between groups with $p < 0.05$ were considered statistically significant.

Results

cDNA sequences for *igf1* and *igf2*

The full-length cDNAs of *igf1 and igf2* were cloned from the liver of YTK and the nucleotide sequences have been deposited in the GenBank database under accession nos. KY405020 and KY405021, respectively. As shown in Fig. 1a, the *igf1* cDNA was composed of 1946 base pairs (bp) which contained a 5′-untranslated region (UTR) of 127 bp, an open reading frame (ORF) of 558 bp encoding preproIGF1 of 185 amino acids (aa), and a 3′-UTR of 1261 bp with the addition of poly (A) tail. The preproIGF1 consisted of 44 aa for the signal peptide, 68 aa for the mature peptide, and 73 aa for the E domain. The B, C, A, and D domains constituting the mature IGF1 protein were composed of 29, 10, 21, and 8 aa, respectively. The calculated molecular mass of mature IGF1 protein was 7.49 kDa, and the isoelectric point was 7.76. Moreover, the six characteristic cysteine residues involved in the formation of the disulfide bonds were conserved (Cys^{B6}, Cys^{B18}, Cys^{A6}, Cys^{A7}, Cys^{A11}, and Cys^{A20}).

For *igf2*, the protein coding region of 648 bp was preceded by a 120-bp 5′-UTR and followed by a 386-bp 3′-UTR (Fig. 1b). The preproIGF2 comprised a total of

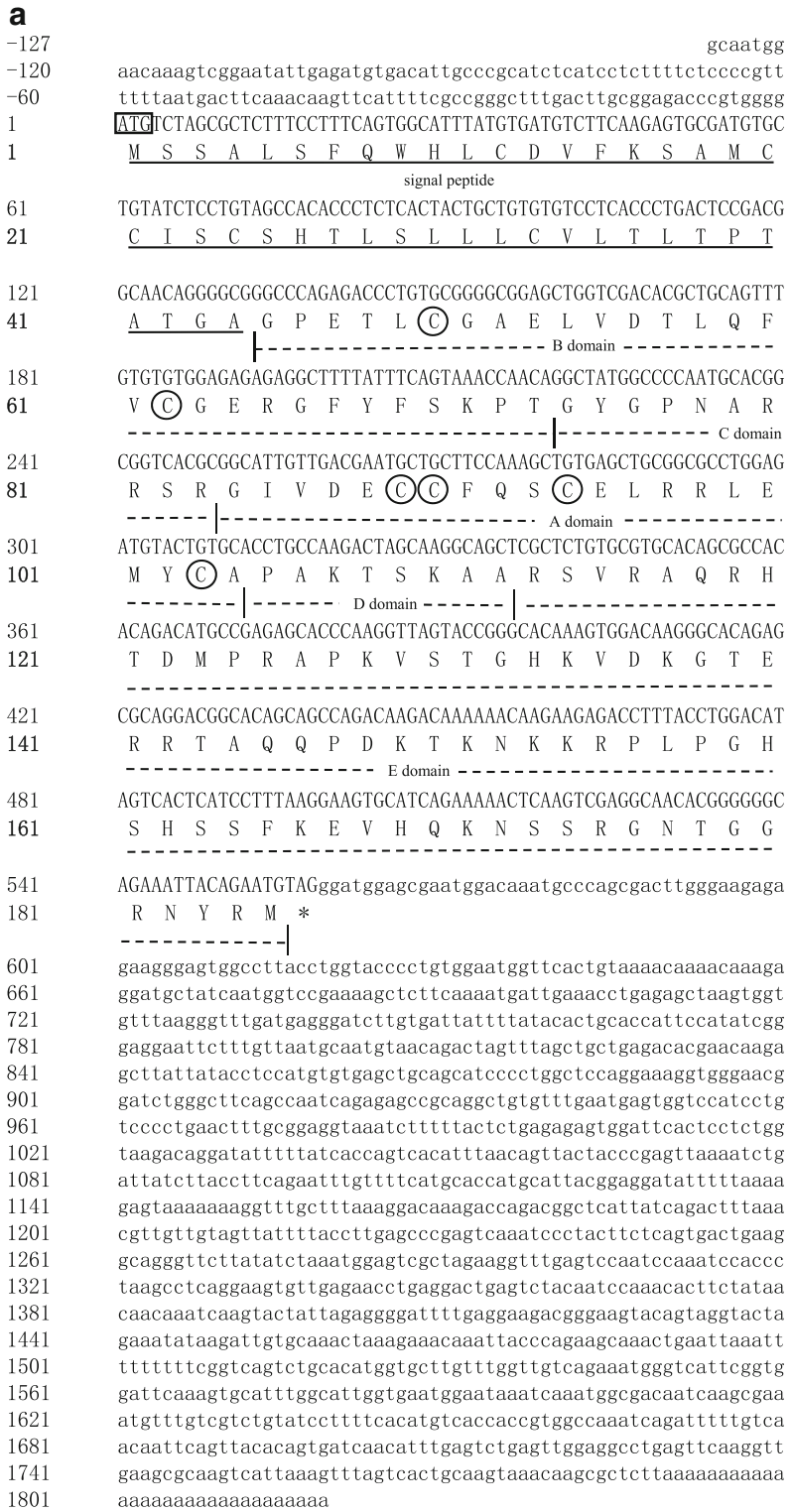


Fig. 1 Nucleotide and deduced amino acid sequences of the cDNAs encoding *igf1* (**a**) and *igf2* (**b**) in yellowtail kingfish. The ORF is indicated by capital letters, and the 5′- and 3′-UTRs are indicated by lowercase letters. The start codon is boxed, and the stop codon is indicated by an asterisk. The signal peptide is underlined, the mature regions consisting of B, C, A, and D domains are indicated, and the six cysteine residues of the mature peptide are indicated by circles

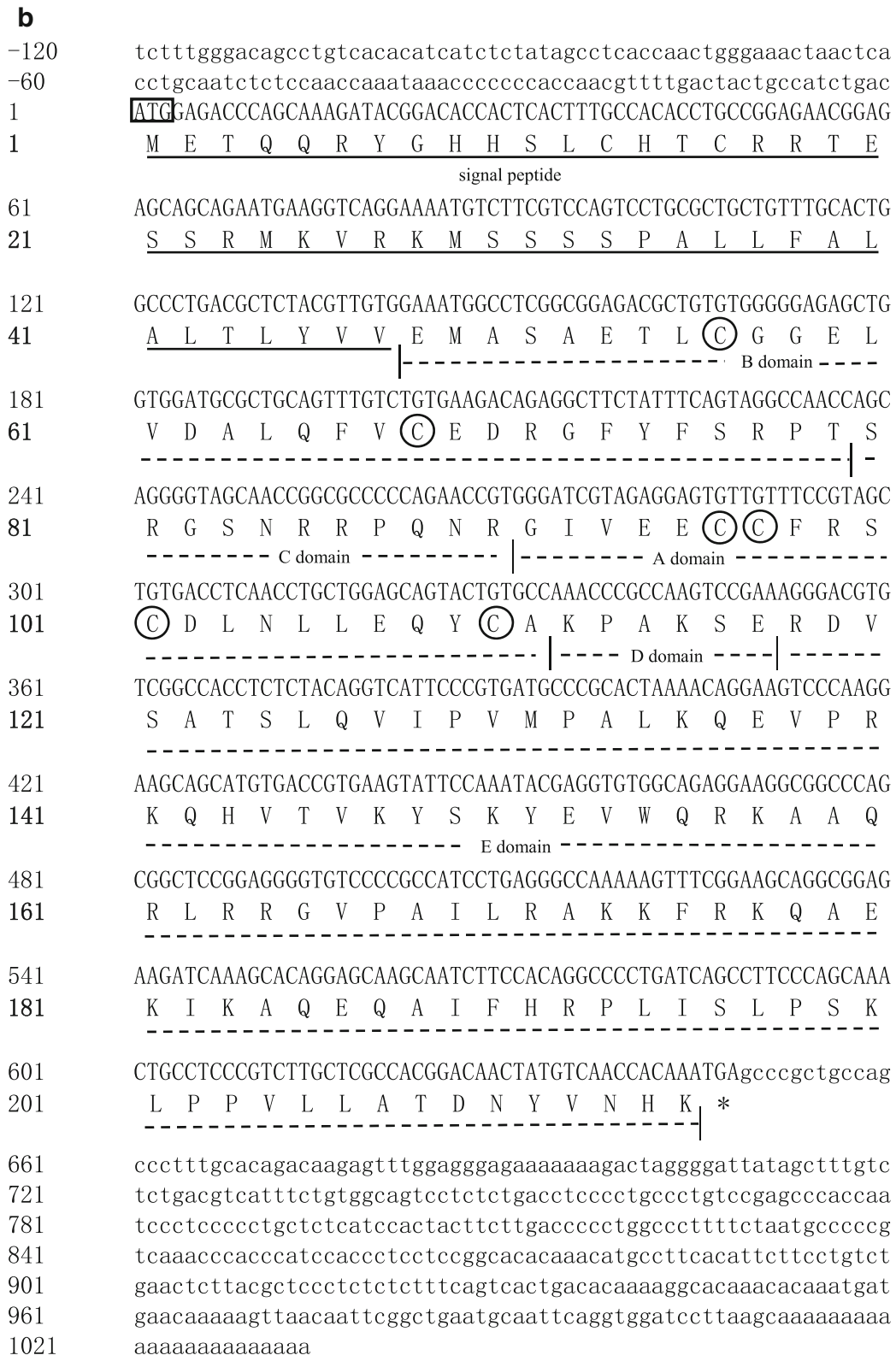


Fig. 1 (continued)

215 aa spanning the signal peptide (47 aa), the mature peptide (70 aa), and the E domain (98 aa). The B, C, A, and D domains constituting the mature IGF2 protein were composed of 32, 11, 21, and 6 aa, respectively. The predicted mature IGF2 protein had a calculated molecular mass and isoelectric point of 7.88 kDa and 5.02, respectively. Similarly, the IGF2 sequence also exhibited the six conserved cysteine residues (Cys^{B9}, Cys^{B21}, Cys^{A6}, Cys^{A7}, Cys^{A11}, and $CysA^{20}$).

Comparison of the IGF1 and IGF2 amino acid sequences

Multiple amino acid sequence alignments of IGF precursors from different teleost species are shown in Fig. 2. The YTK preproIGF1 displayed a high degree of identity with those of grouper (98.38%) and starry flounder (97.28%), followed by tilapia (90.61%), tongue sole (89.73%), tiger puffer (88.95%), and salmonids (85.95–88.44%). However, the sequence homology dropped to relatively lower levels when compared to the counterparts reported in other teleosts (75.97–80.50%). Notably, the C domain of YTK IGF1 had a 2-aa deletion, which was also observed in grouper, tilapia, tongue sole, starry flounder, and tiger puffer compared with other fish (Fig. 2a).

Similarly, the YTK preproIGF2 revealed a high degree of identity with those of grouper (95.81%) and starry flounder (94.42%), followed by tongue sole (91.04%), tilapia (90.70%), tiger puffer (88.37%), and salmonids (83.18–83.64%). However, the sequence homology dropped to relatively lower levels when compared to the counterparts reported in other teleosts (55.72–78.30%).

Phylogenetic analysis of IGF proteins

A phylogenetic tree of the YTK IGF1 and IGF2 with previously identified IGF sequences was constructed (Fig. 3), demonstrating that the IGFs were divided into three distinctive branches. Phylogenetically, YTK IGF1 was closely related to teleost IGF1 clade, less related to the IGF1 proteins reported in tetrapods, and distally related to the IGF2 and IGF3 families. Similarly, YTK IGF2 was closely related to teleost IGF2 clade, less related to the IGF2 proteins reported in tetrapods, and distally related to the IGF1 and IGF3 families.

Tissue distribution of *igf1* and *igf2* mRNAs

To gain insight into the potential physiological roles of IGF1 and IGF2, the gene expression patterns of *igf1* and *igf2* in various tissues were determined by real-time quantitative PCR. As shown in Fig. 4a, a high *igf1* expression was found in the gonad and liver, followed by pituitary and eye, and a low expression in other tissues. On the other hand, *igf2* transcripts were most abundant in the gill and liver, to a lesser extent in the intestine, gonad, and eye. Lower *igf2* mRNA levels were detected in the kidney, brain, and other tissues (Fig. 4b).

Variable expression of *igf1* and *igf2* mRNAs during embryogenesis

The embryonic development of YTK was divided into 18 stages (Fig. 5), and the temporal expression of *igf1* and *igf2* mRNAs during different stages was analyzed (Fig. 6). For all stages of YTK embryogenesis, both *igf1* and *igf2* transcripts were detected with variations in the level of expression. Overall, *igf1* transcript gradually increased over developmental time and reached its peak at the morula stage, followed by an evident decrease afterwards, remaining depressed from the mid-gastrula stage to the end of embryogenesis (Fig. 6a). In contrast, *igf2* mRNA levels remained unchanged before the low blastula stage and then increased dramatically, reaching the top at the early gastrula stage. Subsequently, a remarkable drop was observed and *igf2* gene remained relatively high level to the end of embryogenesis (Fig. 6b).

Discussion

In the current study, we cloned the full-length cDNAs for *igf1* and *igf2* from the liver of YTK and investigated their tissue distribution and expression profiles during embryonic development. YTK IGF1 and IGF2 mature proteins consisted of 68 and 70 aa, respectively. Both IGFs of YTK contained all the features of IGF peptides with B, C, A, and D domains and the conservation of the six cysteine residues involved in the tertiary structure. Sequence alignment revealed that the YTK IGF1 and IGF2 showed a high sequence identity (> 90%) with the counterparts of orange-spotted grouper (Pedroso et al. 2006; Yang et al. 2015), starry flounder (Xu et al. 2015), and

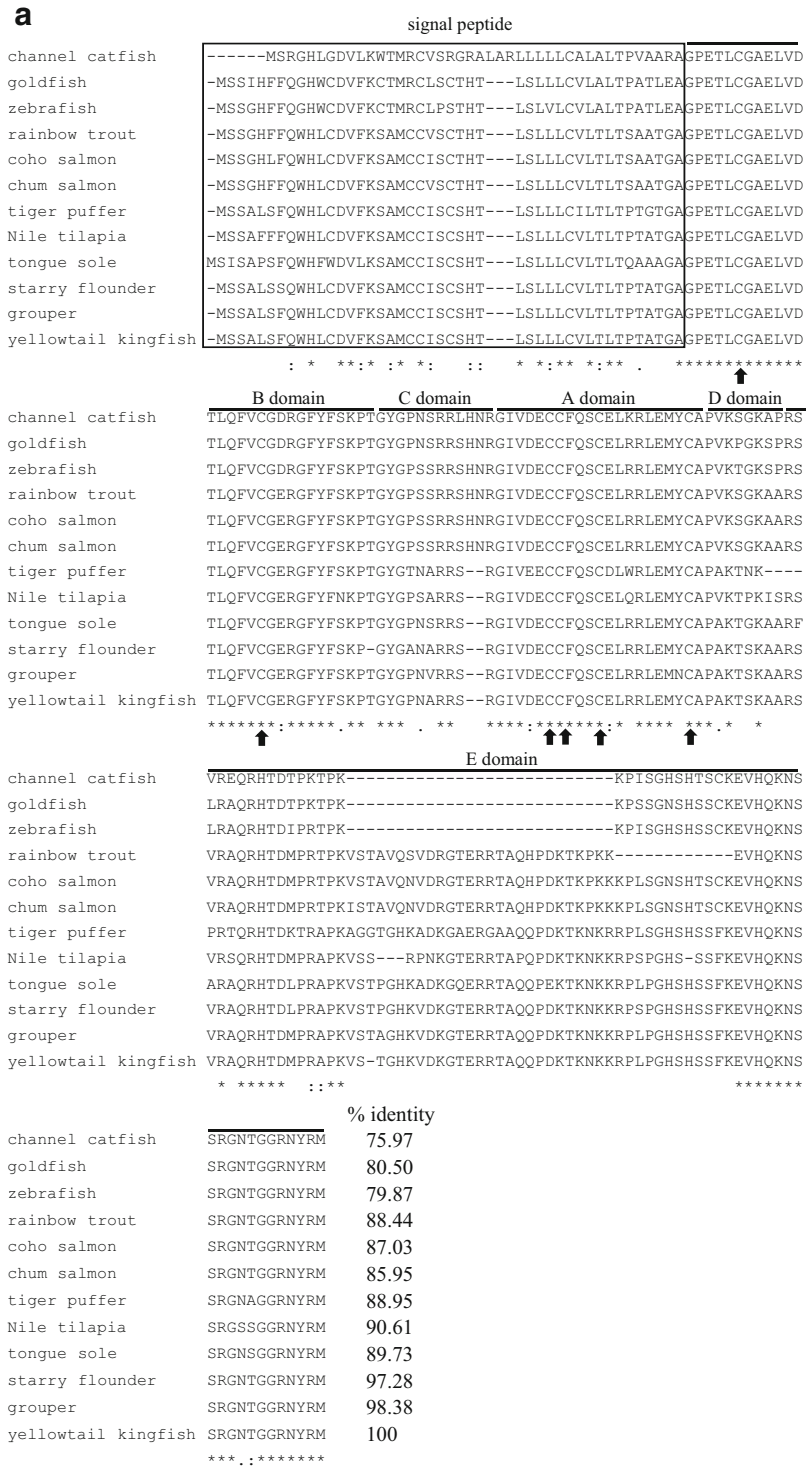


Fig. 2 Alignment of the amino acid sequences of IGF1 (**a**) and IGF2 (**b**) in different species. Gaps introduced in some sequences to maximize the alignment are indicated by hyphens. Identical sequences are indicated by asterisks. Commas denote conserved amino acids and colons indicate highly conserved amino acids. The signal peptide is boxed, the mature regions consisting of B, C, A, and D domains are indicated, and the six cysteine residues of the mature peptide are indicated by arrows

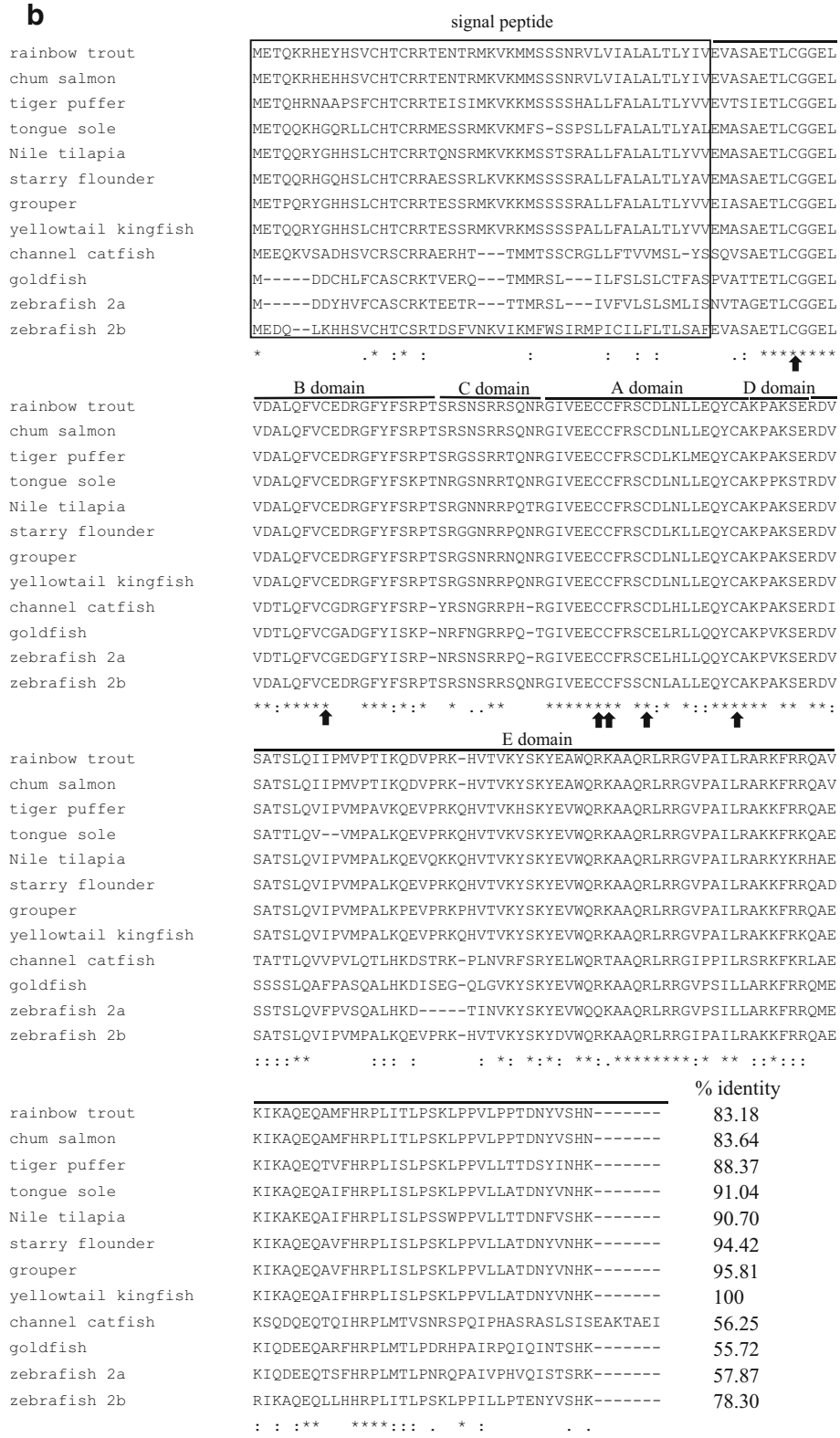
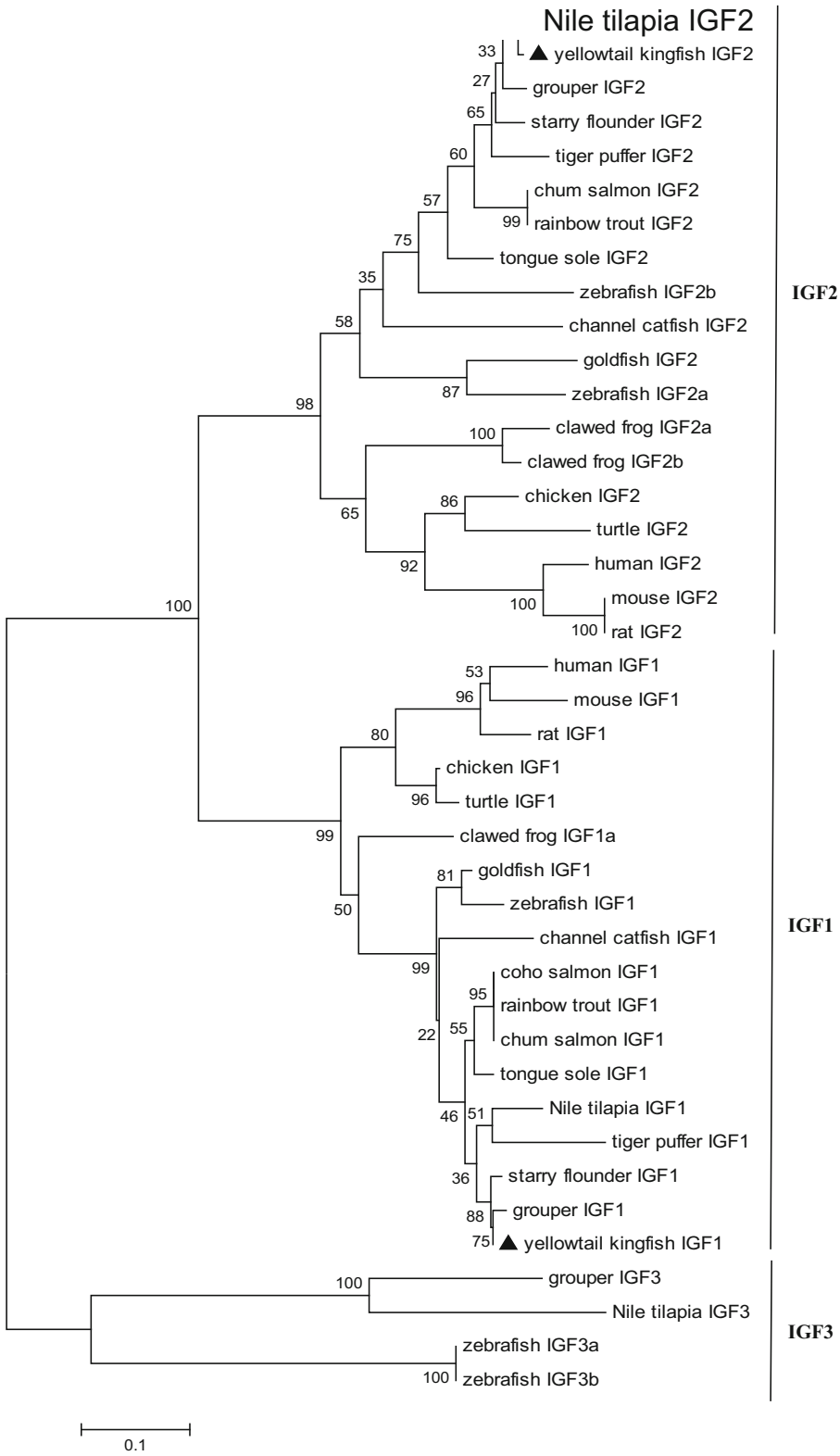


Fig. 2 (continued)


Nile tilapia IGF2
yellowtail kingfish IGF2
grouper IGF2
starry flounder IGF2
tiger puffer IGF2
chum salmon IGF2
rainbow trout IGF2
tongue sole IGF2
zebrafish IGF2b
channel catfish IGF2
goldfish IGF2
zebrafish IGF2a
clawed frog IGF2a
clawed frog IGF2b
chicken IGF2
turtle IGF2
human IGF2
mouse IGF2
rat IGF2
IGF2
human IGF1
mouse IGF1
rat IGF1
chicken IGF1
turtle IGF1
clawed frog IGF1a
goldfish IGF1
zebrafish IGF1
channel catfish IGF1
coho salmon IGF1
rainbow trout IGF1
chum salmon IGF1
tongue sole IGF1
Nile tilapia IGF1
tiger puffer IGF1
starry flounder IGF1
grouper IGF1
yellowtail kingfish IGF1
IGF1
grouper IGF3
Nile tilapia IGF3
zebrafish IGF3a
zebrafish IGF3b
IGF3
0.1

◀ **Fig. 3** Phylogenetic analysis of IGF proteins in vertebrates. The phylogenetic tree was constructed by MEGA 6.06 using the neighbor-joining method with 1000 bootstrap replicates. The number shown at each branch indicates the bootstrap value (%). GenBank accession numbers are as follows: human IGF1 (AAI48267), IGF2 (AAA60088); rat IGF1 (P08025), IGF2 (AAB95624); mouse IGF1 (AAH12409), IGF2 (AAH53489); chicken IGF1 (NP_001004384), IGF2 (NP_001025513); turtle IGF1 (AET11881), IGF2 (XP_006134977); clawed frog IGF1a (NP_001156865), IGF2a (NP_001082128), IGF2b (NP_001085129); goldfish IGF1 (ADD79963), IGF2 (ACJ37294); zebrafish IGF1 (NP_571900), IGF2a (NP_571508), IGF2b (NP_001001815), IGF3a (ADO16598), IGF3b (ADO16599); channel catfish IGF1 (AAZ28918), IGF2 (NP_001187875); tongue sole IGF1 (ACM43291), IGF2 (ACM43292); starry flounder IGF1 (AGN90991), IGF2 (AIY31586); coho salmon IGF1 (P17085); rainbow trout IGF1 (NP_001118168), IGF2 (NP_001118169); chum salmon IGF1 (AAC18833), IGF2 (CAA65862); tiger puffer IGF1 (BAG75453), IGF2 (CAA17123); yellowtail kingfish IGF1 (KY405020), IGF2 (KY405021); grouper IGF1 (AAS01183), IGF2 (AAS58520), IGF3 (AML84199); Nile tilapia IGF1 (ABY88872), IGF2 (ABY88873), IGF3 (ABY88870)

tilapia (Reinecke et al. 1997), respectively. The high degree of identity observed between the different IGF sequences suggests the importance of these peptides for growth and development in teleosts (Reinecke 2010b; Reinecke et al. 2005; Wood et al. 2005). It should be noted that the difference in length of mature IGF1 peptides among species is due specifically to the presence/absence of two amino residues in the C domain, a divergence that occurs at the ordinal level within the teleost lineage (Wood et al. 2005). Specifically, mature IGF1 peptides in Cypriniformes, Salmoniformes, and Siluriformes possess histidine and asparagine residues at positions 39 and 40, respectively (position 1 is designated as the first residue of the mature peptide). In contrast, these residues are absent from the C domain of IGF1 in those Perciformes, Tetraodontiformes, and Pleuronectiformes (Wood et al. 2005).

In addition to IGF1 and IGF2, a third form of IGF (IGF3) with a similar tertiary protein structure has only been identified in some fish species, including common carp (Song et al. 2016), orange-spotted grouper (Yang et al. 2015), tilapia, and zebrafish (Wang et al. 2008). The pronounced expression of *igf3* mRNA in the gonads of adult and developing tilapia highlights the

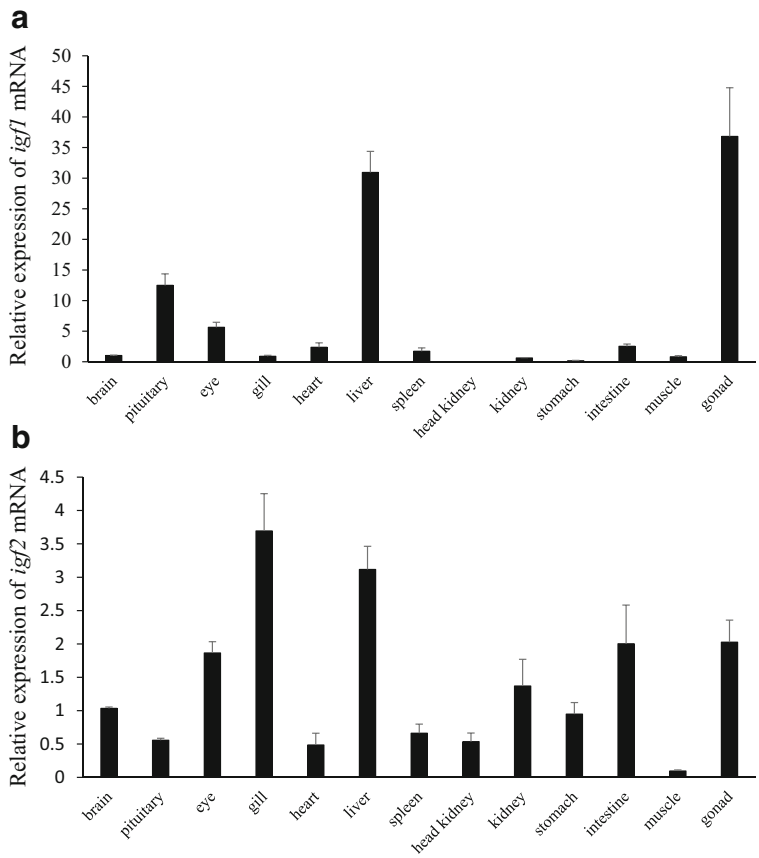


Fig. 4 Relative expression of *igf1* (**a**) and *igf2* (**b**) mRNAs in various tissues of yellowtail kingfish. Data were normalized to the abundance of 18S RNA expressed in the same tissue and presented as the mean ± SEM ($n = 3$)

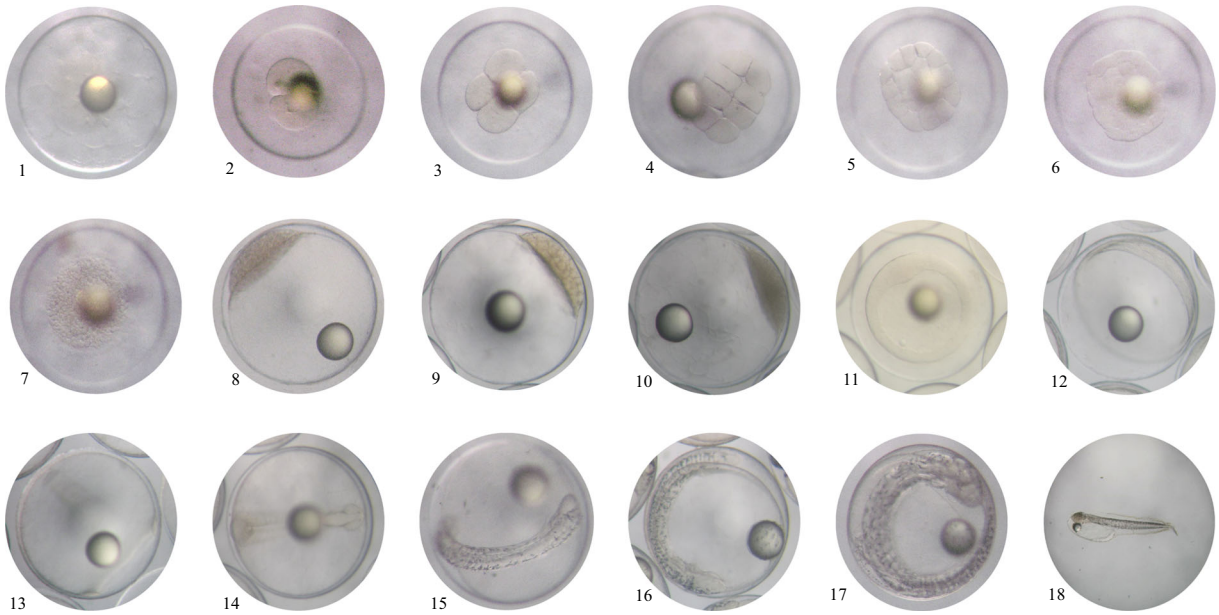


Fig. 5 Embryonic development stages of yellowtail kingfish: (1) fertilized egg; (2) 2-cell; (3) 4-cell; (4) 8-cell; (5) 16-cell; (6) 32-cell; (7) multi-cell; (8) morula; (9) high blastula; (10) low blastula; (11) early gastrula; (12) mid-gastrula; (13) late gastrula; (14) appearance of embryo; (15) embryo encompassing yolk 50%; (16) embryo encompassing yolk 70%; (17) embryo encompassing yolk 100%; (18) newly hatched larva

importance of this novel IGF in teleost gonadal development and reproduction (Berishvili et al. 2010; Wang et al. 2008). Indeed, preliminary studies have indicated that IGF3 can regulate expression of genes encoding steroidogenic enzymes and key transcription factors in the gonads of tilapia (Li et al. 2012). Moreover, IGF3 exerted a potent action in stimulating oocyte maturation and also mediated the action of LH on oocyte maturation in zebrafish (Li et al. 2011, 2015). During development of tilapia, the *igf3* gene was significantly upregulated in male but downregulated in female gonad, and 17α-ethinylestradiol treatments resulted in significant downregulations of *igf3* mRNA in testis while ovarian *igf3* mRNA did not respond, suggesting that IGF3 may be involved in reproduction of fishes most likely in the male gonad only (Berishvili et al. 2010). On the other hand, in the gonad development stages of common carp, *igf3a* mRNA expression was highest in the maturity and recession stage of the ovary, and decline phase of the testis, while *igf3b* was highest in the recession and fully mature periods of the ovaries and testes, respectively. Notably, 17β-ethinylestradiol treatment increased both ovary and testis *igf3* mRNA expression (Song et al. 2016). Further investigation is warranted to clarify whether the novel IGF3 form exists in YTK and the potential physicological functions as observed in other teleosts.

IGFs are primarily produced in the liver although they are also synthesized in most extrahepatic tissues in a species-specific manner. Consistent with previous studies in common carp (Tse et al. 2002; Vong et al. 2003), coho salmon (Pierce et al. 2004), tilapia (Caelers et al. 2004), Senegal sole (Funes et al. 2006), redbanded seabream (Ponce et al. 2008), pejerrey (Sciara et al. 2008), giant grouper (Dong et al. 2010), tongue sole (Ma et al. 2011), starry flounder (Xu et al. 2015), Japanese amberjack (Higuchi et al. 2016), and white seabream (Perez et al. 2016), a high amount of *igf1* transcripts were observed in the liver of YTK, suggesting that the liver plays a pivotal role in IGF1 production and processing in teleosts. In addition, a substantial degree of YTK *igf1* mRNA expression was also evident in the gonad, suggesting potential paracrine/autocrine actions of local IGF1 involved in the gonadal development (Higuchi et al. 2016; Reinecke 2010b; Xu et al. 2017; Yuan et al. 2018). On the other hand, *igf2* mRNA was mostly expressed in the gill, which was in line with previous reports in Senegal sole (Funes et al. 2006), redbanded seabream (Ponce et al. 2008), Japanese amberjack (Higuchi et al. 2016), and white seabream (Perez et al. 2016), indicating a possible role of this hormone in osmoregulation. Similar to the situation in tilapia (Caelers et al. 2004), Japanese eel (Moriyama

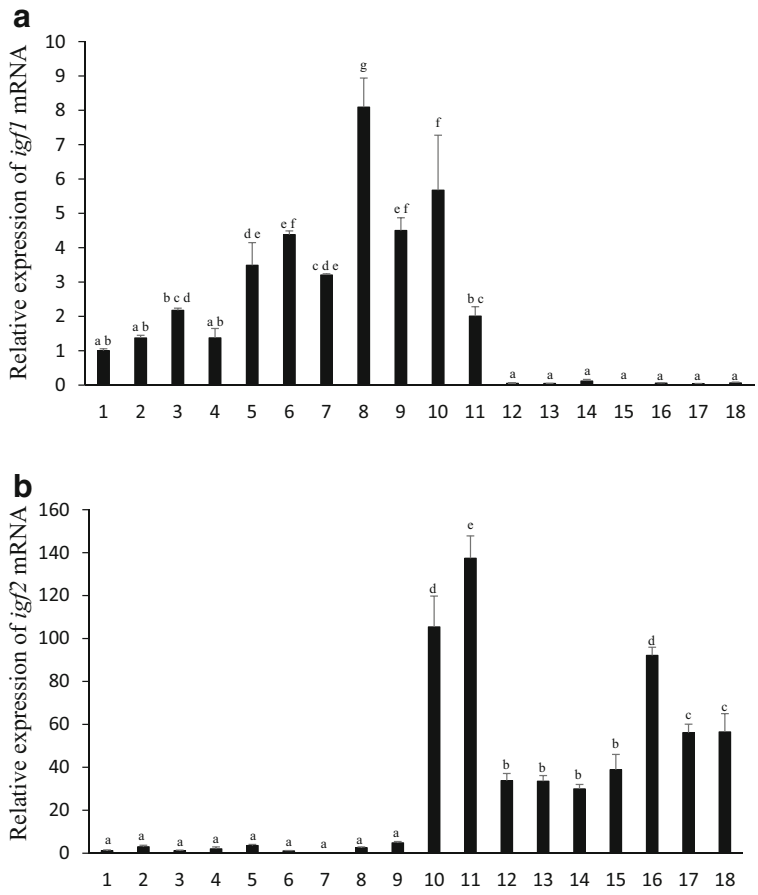


Fig. 6 Expression of *igf1* (**a**) and *igf2* (**b**) mRNAs during embryonic development of yellowtail kingfish. Data were normalized with the abundance of 18S RNA expressed in the same stage of embryo and are presented as the mean ± SEM ($n = 3$). Groups with different letters are significantly different from each other ($p < 0.05$; ANOVA followed by Duncan's multiple range test). Please refer to Fig. 5 for the details of embryonic stages

et al. 2008), starry flounder (Xu et al. 2015), and Japanese amberjack (Higuchi et al. 2016), a large amount of *igf2* transcripts were also observed in the liver of YTK. Furthermore, detectable amounts of *igf2* mRNAs were measured in all the other tissues examined, consistent with the situation in other teleosts but in contrast to mammals (Funes et al. 2006; Higuchi et al. 2016; Moriyama et al. 2008; Perez et al. 2016; Reinecke et al. 2005; Wang et al. 2008; Yang et al. 2015). Taken together, these differences in the expression level of *igf* mRNAs may simply be due to species variation, and other parameters such as nutritional status and environmental factors, as well as developmental stage of individuals, may also account for the differences (Duan 1998; Reindl and Sheridan 2012; Reinecke 2010a).

Several studies have shown that the IGF system plays an essential role during embryogenesis of fish with distinct temporal-spatial expression of *igf* genes in starry flounder (Xu et al. 2015), zebrafish (Li et al. 2014), hybrid and channel catfish (Peterson et al. 2005), rabbitfish (Ayson et al. 2002), and gilthead seabream (Perrot et al. 1999). Interestingly, both *igf1* and *igf2* mRNAs were detected in unfertilized eggs and during embryogenesis of starry flounder (Xu et al. 2015), gilthead seabream (Perrot et al. 1999), hybrid and channel catfish (Peterson et al. 2005), suggesting that these mRNAs appear to be products of both maternal and embryonic genomes. Unlike mammalian embryos, fish embryos develop outside the maternal body and thus rely on growth factors that are maternally stored (Perrot et al. 1999). However, *igf2* but not *igf1* mRNA was expressed in unfertilized eggs and in all stages of embryogenesis of rabbitfish (Ayson et al. 2002). Differences in expression patterns of *igf1* mRNA in embryonic development of teleosts may be related to differences among species.

In the current study, both *igf1* and *igf2* mRNAs were detected in all stages of YTK embryogenesis and seemed to be developmentally regulated. *igf1* mRNA increased significantly during the early development of YTK embryos, while a high level of *igf2* mRNA was observed during the late developmental stages. This

temporal pattern of expression was generally similar to that of starry flounder, suggesting that IGFI might play a very important role in cell proliferation during the cleavage period (Xu et al. 2015). Indeed, IGF1 stimulated zebrafish embryonic cell proliferation by activating the MAPK and PI3K pathways (Pozios et al. 2001) and injection of *igf1* mRNA into zebrafish blastomeres (1–4 cell stage embryos) resulted in a greatly expanded development of anterior structures at the expense of trunk and tail (Eivers et al. 2004). On the other hand, *igf2* expression was higher than *igf1* expression in rainbow trout and rabbitfish embryos, suggesting IGF2 was probably more important than IGF1 during embryogenesis (Ayson et al. 2002; Gabillard et al. 2003; Greene and Chen 1999). Targeted *igf2* gene knockdown in zebrafish revealed distinct intraembryonic functions (White et al. 2009) and IGF2 can sustain the self-renewal of embryonic stem cell line and blastomeres of medaka (Yuan and Hong 2017). In addition, IGF1 receptor-mediated signaling was required for the proper growth, development, and survival of zebrafish embryos (Schlueter et al. 2007). Taken together, these data indicate that the developmental regulation of *igf1* and *igf2* appears to be specific to species and gene, and these two peptides may exert differential functions and coordinate with each other in the regulation of embryo development of teleosts.

It is worth mentioning that the local production of IGF in multiple organs of teleosts during development indicates paracrine/autocrine actions of IGF involved in organ-specific functions. For instance, the development of *igf1* gene expression in tilapia pituitary revealed that IGF1 may regulate synthesis and release of pituitary hormones (Berishvili et al. 2006b; Moret et al. 2008). Similarly, the appearance and distribution of *igf1* and *igf3* mRNAs and/or peptides during the early development of tilapia gonads suggest an important physiological impact of local IGF in the formation and differentiation of gonads (Berishvili et al. 2010, 2006a; Wang et al. 2008). However, these actions have yet to be fully elucidated in YTK. Accordingly, clarifying organ-specific expression of *igf1* and *igf2* during early development of YTK will be an interesting topic in our ongoing study.

Conclusions

In summary, we have cloned *igf1* and *igf2* cDNAs from YTK, confirming high sequence identity among species, which suggests an important role of these growth factors conserved during evolution. In addition, the spatio-temporal expression profiles of *igf1* and *igf2* mRNAs were also examined. Overall, the present study contributes to the knowledge of the IGF system in the embryonic development of yellowtail kingfish, of which is a crucial period for developing successful farming of this species.

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