The Profile of Growth Hormone Gene Expression in Extrapituitary Tissues of *Lepomis cyanellus*

CAO Yun-chang¹, WEN Hong-bo¹, LI Wen-sheng², LIN Hao-ran²

¹ School of Life Sciences & Biotechnology, Nanhua University, Hengyang 421001, China; 2. Institute of Aquatic Economical Animal & Guangdong Provinical Key Laboratory for Aquatic Economical Animal, Zhongshan University, Guangzhou 510275, China

Abstract: By semi-quantitative RT-PCR method and Southern blotting technology, growth hormone (GH) gene expression was determined in extra-pituitary tissues of green sunfish, *Lepomis cyanellus*. After the condition of semi-quantitative RT-PCR was established, 12 tissues of 6 months old (larvae stage) and 1 year old (mature stage) male *L. cyanellus* were detected. The results showed that GH gene was expressed in muscle, gonad, gall, heart, brain, and kidney in different level besides pituitary gland, and that no GH expression was detected in spleen, liver and stomach. Unexpectedly and interestingly, GH expression in muscle of adult fish was distinctly higher than that of sexually immature fish. In addition, the transcript levels of GH mRNA in the gonad of larvae and adult fishes were high. The data presented here provide the first systematic report of universal GH gene expression in extra-pituitary tissues in fish, suggesting that GH through autocrine or paracrine pathway exerted its possible important local physiological function during development and growth of *L. cyanellus*.

Key words: *Lepomis cyanellus*; Growth hormone; Semi-quantitative RT-PCR; Gene expression

1.2.2 Genebank accession code: AY530822[4] for cDNA

1.2.3 Genebank accession code: AY530822[4] for mRNA

1.2.4 Genebank accession code: AY530822[4] for DNA

2.1 The expression of GH mRNA in the pituitary gland was observed in the liver, muscle, and other organs. sqRT-PCR and RT-PCR Southern blotting were used to detect GH mRNA expression.

Materials and Methods

1.1 Experimental Fish and Reagents

Experimental fish were blue sunfish obtained from the Guangdong Provincial Fengnan Fishery Breeding Station. Fish of different ages were selected: 6-month-old and mature (male and female) blue sunfish for each group. The samples were rinsed with DEPC water to remove residual blood and stored in liquid nitrogen.

Extraction reagents were purchased from F.74&“GH% company; sqRT-PCR reagents were purchased from F.74&“GH% company; enzymes were purchased from LIF company. Georgian “Stary” biotest; “Stary” biotest; “Stary” biotest; F.74&“GH% company; other reagents were purchased from domestic pure reagents.

Method

1.2.1 RNA Extraction and Reverse Transcription

Blue sunfish samples were homogenized using a one-use disposable plastic syringe in liquid nitrogen, and the RNA was extracted using trizole. RNA was reverse transcribed using Oligo-dT primer and reverse transcriptase.

1.2.2 Amplification of GH mRNA

Amplification primers were designed based on the blue sunfish GH mRNA sequence (BUVQ*W00) and were located in the open reading frame of the GH mRNA gene. The amplification product was 346 bp.

正义链:

反义链:

1.2.3 sqRT-PCR: 346 bp

1.2.4 Southern blotting: 346 bp

1.2.5 The 346 bp amplification product was analyzed by agarose gel electrophoresis. The probe was synthesized using the digoxigenin-labeled method. The probe was 346 bp long and synthesized according to the kit instructions.

1.2.6 After denaturing, the PCR products were transferred to nylon membranes. The membranes were pre-hybridized, hybridized, washed, and closed with digoxigenin antibodies, and then subjected to chemical luminescence detection on a British M6.+%.% company product.

1.2.7 Other reagents were purchased from domestic pure reagents.

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3 RNA

mRNA

mRNA

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Northern blotting

mRNA

mRNA

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Fig. 2  Relative expression quantity C and the results of RT-PCR Southern A B of GH expression products in tissues of 1 year old Leponis cyanellus.

M 100 bp DNA ladder marker Sp Stomach Hy Hypothalamus Sp Spleen L Liver Kidney Intestine Brain Gill Gill Heart Go

Molar M Mu Muscle P Pituitary

2 1 GH mRNA RT-PCR Southern A B

Harvey et al. 2000 Hull et al. 1996 Harvey et al. 1998 GH mRNA


RT-PCR Southern

18S rRNA

6 Southern GH mRNA GH

18S rRNA
鳃、心脏、肾脏；而脾脏、肝脏、胃个组织均未检测到表达。此外，月龄的肠道组织未检测到表达，但年龄（性成熟）时却检测到了表达；月龄的下丘脑检测到了表达，而到性成熟期时却检测不到表达。值得注意的是，月龄的肌肉组织的相对表达量为低水平表达，而到性成熟期时相对表达量明显提高，仅次于垂体的相对表达量。我们推测，这可能是因为在幼鱼期垂体中GH的合成和分泌非常旺盛，通过—轴刺激肝脏合成的IGF水平比较高，从而抑制了肌肉细胞中GH的表达；而到了成鱼期后，垂体合成和分泌的GH水平相对降低，鱼体肌肉组织的合成代谢减弱，此时需要肌肉组织表达生长激素以产生一种代偿作用所导致。此外还发现，在性腺组织（精巢）的两个时期，GH都维持在较高的表达水平。据此作者认为，这些在垂体外组织中合成的GH可能是直接作用于自身细胞或相邻的细胞，以自分泌或旁分泌的方式发挥其重要的生理功能，如性腺中合成的GH直接作用于性腺刺激生殖细胞的发育，肌肉细胞中合成的GH通过—轴或其他途径对肌肉细胞的代谢进行调节。

参考文献: