Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Transcription of blunt snout bream (*Megalobrama amblycephala*) *HIF3α* and its localization in the nucleus under both normoxic and hypoxic conditions

Ziyin Liu [1], Xinyu Zhao [1], Xiayun Jiang [**], Shuming Zou [*]

Genetics and Breeding Center for Blunt Snout Bream, Key Laboratory of Freshwater Aquatic Genetic Resources, Ministry of Agriculture, Shanghai Ocean University, Huchenghuan Road 999, Shanghai, 201306, China

ARTICLE INFO

Article history:
Received 11 April 2018
Accepted 12 April 2018
Available online xxx

Keywords:
HIF3α
Normoxia
Hypoxia
Nuclear localization
Nucleus
NLS

ABSTRACT

Although hypoxia-inducible factor (HIF) 1α and 2α function as master regulators of the transcriptional response to hypoxia, the function of HIF3α and its responses to hypoxic stress remain unclear in teleost fish. Here, we characterized the *HIF3α* cDNA in hypoxia-sensitive blunt snout bream (*Megalobrama amblycephala*), with 3059 bp length, consisting of an open reading frame (ORF) encoding 643 amino acid residues. Blunt snout bream *HIF3α* mRNA was stably expressed during stages of embryonic development and in adult tissues. After a 4 h hypoxia stress, *HIF3α* mRNA of the juvenile fish was significantly upregulated in the liver, brain, and kidney, and restored to the pretreatment levels after a 24 h recovery. When tagged with enhanced green fluorescent protein (EGFP) and transfected into cultured HeLa cells, blunt snout bream HIF3α was mainly distributed in the nucleus under normoxia. Treatment of the cells with $CoCl_2$ to mimic hypoxic conditions showed that there was no effect about the nuclear localization of HIF3α but a statistically significant increase in HIF3α protein levels. A nuclear localization signal (NLS) sequence at the C-terminus of HIF3α may exert positive effects in the process of nuclear localization. These results suggest that blunt snout bream *HIF3α* could be involved in different physiological functions under normoxia and hypoxia conditions.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Hypoxia-inducible factors (HIFs) comprise a family of evolutionarily conserved transcriptional regulators that affect the homeostatic response to low oxygen tension and play key roles in coordinating the cellular response to hypoxia [1]. The heterodimeric HIFs composed of a labile hypoxia-regulated α subunit, the so called HIF1α, 2α, or 3α, and a constitutive β subunit [2,3]. Under normoxia, HIFαs are hydroxylated by proline hydroxylase (PHD) at proline residues in their oxygen-dependent degradation domain (ODD), this leads to their binding to the E3 ubiquitin ligase von Hippel-Lindau protein (pVHL) and targeting to the proteasome for degradation [4–7]. Simultaneously, a conserved asparagine residue in the C-TAD is hydroxylated by factor inhibiting HIF-1 (FIH) [8]. Under hypoxic conditions, HIFαs are stably accumulated and transported into the nucleus to form functional transcription complexes with HIFβ, combining with hypoxia response elements (HRE) to regulate target genes [6].

The structure and function of HIFαs are important to explain the molecular mechanism of response to hypoxia, however, compared with the extensive understanding of HIF1α and HIF2α, our knowledge of HIF3α is limited [9]. Although there are several studies on the distribution and regulation of HIF3α and its responses to hypoxia in mammals and humans [4,10], the role of HIF3α and the corresponding information in fish under hypoxia remains to be determined. HIF3α has only been described in few fish species recently [7,11–16]. Available evidence suggests that the *HIF3α* gene generates multiple HIF3α variants owing to the utilization of different promoters, different transcription initiation sites, and alternative splicing. These different *HIF3α* variants have different and even opposite functions [14,16,17].

Blunt snout bream is an important commercial fish in China and a hypoxia-sensitive species. Massive die-off as a result of hypoxia is a concern due to the low oxygen levels present during

* Corresponding author.
** Corresponding author.
E-mail addresses: jiangxy@shou.edu.cn (X. Jiang), smzou@shou.edu.cn (S. Zou).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.04.099
0006-291X/© 2018 Elsevier Inc. All rights reserved.

breeding. Therefore, fundamentally understanding the hypoxia response mechanism of blunt snout bream *HIF3α* can strengthen breeding selection and improve the economic benefit of fishery production. In this study, we demonstrate that HIF3α is a member of the bHLH family, *HIF3α* mRNA is stably expressed during stages of embryonic development and in adult tissues, and HIF3α protein is distributed in the nucleus of HeLa cells transfected with reporter plasmids.

2. Materials and methods

2.1. Sequence analysis

In this study, the full-length cDNA of the blunt snout bream *HIF3α* was obtained in Genebank (GU363500.1) from NCBI (https://www.ncbi.nlm.nih.gov/). The alignment of the putative amino acid sequences of the HIFα protein was performed using Clustal X. Gene structure was analyzed using the Genscan program (http://genes.mit.edu/GENSCAN.html).

2.2. Experimental fish

All experiments were conducted following guidelines approved by the Shanghai Ocean University Committee on the Use and Care of Animals. Embryos were generated by natural crosses of blunt snout bream and provided by the Genetics and Breeding Center of the Ministry of Agriculture for Blunt Snout Bream, Shanghai, China. Approximately 100–200 fertilized eggs were plated in each Petri dish (15 cm diameter). Embryo development was carried out at 22 °C. To maintain normoxic dissolved oxygen (DO) values at 7.0 ± 0.5 mg/L during embryogenesis, water in the Petri dish was replaced every 2–3 h with well-aerated water, and supplemented with 0.003% (w/v) 2-phenylthiourea to inhibit embryo pigment formation. Adult fish were anaesthetized for 5 min in well-aerated water containing a high concentration (1 g/L) of MS-222. Tissue samples (brain, intestine, liver, gill, heart, eye, kidney, muscle, spleen, testis, and ovary) were immediately collected, frozen in liquid nitrogen, and stored at −80 °C until use.

2.3. Plasmids, cell culture and transfection

Plasmid EGFP-C1, which contains EGFP driven from a CMV promoter, was obtained from Clontech. The pCS2-HIF3α-EGFP plasmid was constructed by inserting blunt snout bream *HIF3α* coding sequence into the pCS2-EGFP expression vectors and stored in our laboratory at −80 °C, which was tagged with EGFP at the C-terminus. The primer pairs used here were HIF3α-F: GTGGATC-CACCATGGTGAACTCAGTGATTAAGAGG and HIF3α-R: CAATCGAT-GAGTAAGGGACGACATGGTTTC. HeLa cells (from CCTCC) were grown in medium M199 (GIBCO) supplemented with 10% FBS (GIBCO) and penicillin and streptomycin, under normoxia (21% O_2, 5% CO_2, 74% N_2) at 37 °C in a constant temperature cell incubator. Cells were plated onto 24-well plates ($0.5–2 \times 10^5$ cells/well) 24 h overnight to 50% confluency prior to transfection. We transiently transfected EGFP-C1 and pCS2-HIF3α-EGFP plasmids into HeLa cells using ViaFect Transfection Reagent (Promega, Madison, WI, USA). For each transfection, 3 μl of TransFast™ Reagent was incubated for 5 min in 50 μl serum-free medium (OPTI-MEM) before the addition of 0.8 μg of plasmid DNA in 50 μl serum-free medium (total volume of 100 μl). The TransFast™ Reagent/DNA mixture was incubated at 25 °C for 20 min and added directly to the culture in a drop-wise manner before agitation. Then the cells were incubated for 48 h to allow DNA uptake and gene expression. Each experiment was repeated in triplicate.

2.4. Immunocytochemical analysis

After transfection, the cells were fixed with 4% PFA in PBS for 30 min, then washed briefly with PBS and counterstained with 500 μl of DAPI solution (2 μg/ml), which was removed after immunofluorescence staining. After rinsing the cells several times in PBS, images were recorded with a Leica DFC450C digital camera and a Leica DMI3000B microscope with appropriate filters and a digital camera. Finally, the fluorescence patterns were observed and photographed by using a laser scanning cofocal imaging system.

2.5. Quantitative real-time (qRT)-PCR

Total RNA was isolated from different embryostages or adult tissues using TRIzol reagent (Invitrogen, USA). According to the protocol, 1 μg of RNA was reverse-transcribed to single-strand cDNA using SuperScript II reverse transcriptase (Invitrogen, USA) with oligo-dT as primers. For PCR, 2 μL cDNA was diluted to 50 μL in which 0.5 μL *Taq* DNA polymerase (Qiagen,USA), 5 μL buffer solution, 1 μL each of the primers (10 mM), 2 μL dNTP mixtures (2.5 mM), and 38.5 μL ddH_2O were included. The PCR program was as follows: 1 cycle of 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 90 s, and 1 cycle of 72 °C for 5 min and holding at 4 °C. The amplification efficiency (E) of each primer pair was calculated based on the slope of a linear regression from a dilution series of cDNA. Relative expression analyses used the comparative threshold cycle (CT) method using *β-actin* as reference genes and based on the standard curve and normalized to *β-actin* mRNA level [9]. The primer pairs used here were HIF3α-*q*RT-F: GACCGACAGGGACCCGTTAC and HIF3α-*q*RT-R: ATGGTTTCAGCAACAGGTCGC.

2.6. Hypoxia treatments

Ten juvenile fish (~20 g) were transferred into each of three 50-L tanks within a continuous flow system. After 1 week of acclimation, two experimental groups were exposed to severe hypoxic conditions (1.0 ± 0.5 mg/L) for 4 h by nitrogen-filled manipulation [18], while the control group was under normoxic DO conditions (7.0 ± 0.5 mg/L). DO values were monitored continuously using a WTW Multi 340i (WTW, Germany). The hypoxic DO value was set based on the previous observations of the juvenile blunt snout bream [19]. After the exposure period, five fish from each hypoxic treatment and the control group were sampled. The DO levels of the two experimental groups were then adjusted back to normal levels within 1 h. The remaining five fish from each recovery treatment and the control group were sampled 24 h later. Liver, brain, and kidney were immediately excised and frozen in liquid nitrogen, then stored at −80 °C until use.

We used $CoCl_2$ to mimic the hypoxic effect, which is a compound that inhibits PHD activity to block the oxygen-dependent enzymatic degradation of HIF3α [20]. Before microscopy, HeLa cells were transiently transfected with either EGFP-C1 or pCS2-HIF3α-EGFP plasmid and after 24 h, expression was induced for 6 h with either 50, 100, 150, 200, 300, or 400 μM $CoCl_2$ [21]. After the transfection, the HeLa cells were lysed and subjected to Western immunoblotting using the HIF3α antibody as Zhang et al. have described previously [9].

3. Results

3.1. Characterization of blunt snout bream HIF3α

Blunt snout bream *HIF3α* sequence is 3059 bp, consisting of an ORF encoding 643 amino acid residues and belonging to the bHLH

Download English Version:

https://daneshyari.com/en/article/8292920

Download Persian Version:

https://daneshyari.com/article/8292920

Daneshyari.com