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Original Article

Cloning and expression analysis of *hif-2α* gene in cobia (*Rachycentron canadum*) under hypoxia stress

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Abstract: Hypoxia-inducible factor 2α (*hif-2α*) is a critical regulator of hypoxia response and plays a vital role in hypoxia stress in the organism. To understand the regulatory role of *hif-2α* in response to hypoxia stress in juvenile cobia (*Rachycentron canadum*), *hif-2α* was cloned using Rapid Amplification of cDNA Ends (RACE) technology. The full length of *hif-2α* is 4021 bp, with 2634 bp open reading frame (ORF), 5' non-coding region (5'UTR), 285 bp, 3' non-coding region (3'-UTR), 1102 bp, and encoding 877 amino acids. The encoded protein contains the HLH (Helix-loop-helix) domain (amino acids 20-75), the PAS (PER-ARNT-SIM) domain (amino acids 91-157 and 237-303), and the PAC (PAS Associated C-terminal) domain (amino acids 309-352). The results of phylogenetic tree analysis showed that *hif-2α* in cobia clustered with *hif-2α* in *Echeneis naucrates* and were closely related. Real-time fluorescence quantitative PCR (qRT-PCR) was used to analyze the expression of *hif-2α* in nine different tissues of cobia and the expression of *hif-2α* mRNA in the liver and gill under hypoxia stress. The results suggested that the *hif-2α* was expressed in all tissues of the cobia, with higher expression in the liver and gill. Under hypoxia stress, the expression of the *hif-2α* in the liver and gill was tissue-specific. In liver tissues, *hif-2α* expression was significantly higher than that of the control at 14 and 28 days. In gill tissues, *hif-2α* expression decreased at 7, 14, and 28 days and was lowest at 14 day. The results suggest that *hif-2α* plays a vital role in hypoxic stress in cobia and may provide basic information for studying the molecular genetic mechanism of hypoxia tolerance in cobia.

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Introduction

Dissolved Oxygen (DO) is one of the critical environmental factors for the growth and development of aquatic organisms and their healthy survival. Due to the influence of water temperature, weather, eutrophication, and high-density aquaculture, the dissolved oxygen content of water bodies is constantly changing, which can easily lead to hypoxia (Wang et al., 2021a; Lu et al., 2022). Numerous studies have shown that hypoxia stress has adverse impacts on the physiological activities of aquatic animals, including growth retardation, oxidative stress damage, apoptosis, and impaired immune function (Chen et al., 2016; Kim et al., 2021; Wang et al., 2021b; Wang et al., 2022), and these biological processes are often associated with critical genes and their expression in the organism (Abdel-Tawwab et al., 2019; Zhang et al., 2020). Therefore, understanding the molecular characteristics and functions of hypoxia-related genes in aquatic organisms is of potential significance to the health of aquaculture.

Hypoxia-inducible factors (HIFs) are a class of evolutionarily conserved transcriptional regulators that play an essential role in the response of chordates to hypoxia stress (Liu et al., 2018). HIFs consist of α subunits (HIF-αs: HIF-1α, HIF-2α, and HIF-3α) and

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β subunits, of which the α subunits are particularly sensitive to oxygen concentration and play a significant regulatory function (Shen et al., 2010; Zhang et al., 2014; Feng et al., 2019). The α subunit is particularly sensitive to oxygen concentration and plays a significant regulatory role, while the β subunit is insensitive to oxygen concentration (Shen et al., 2010; Zhang et al., 2014; Feng et al., 2019). Hypoxia-inducible factor 2α (Hif-2α), also known as endothelial PAS domain-containing protein 1 (EPAS1), is a newly discovered protein molecule that binds DNA (Liu et al., 2011; Alrezk et al., 2018). HIF-2α is closely associated with diseases such as tumors and pulmonary hypertension and plays a vital role in vascular function, bone marrow hematopoiesis, and energy metabolism (Shen et al., 2010; Deng et al., 2017). The HIF-1α and HIF-2α are similar in molecular structure, DNA binding, and transcriptional activity but differ in the regulation of gene expression and thus play different physiological functions in many hypoxia-related pathologies (Tian et al., 1997; Fan et al., 2018). It has been found that HIF-1α is a significant regulator in acute or short-term hypoxic responses, HIF-2α plays an essential regulatory role mainly in chronic or long-term hypoxic responses (Rytkönen et al., 2013), and HIF-3α can act as a negative regulator of HIF-1α and HIF-2α (Cai et al., 2020).

Currently, some progress has been made in studying the *hif-2α* gene in fish. For example, Rojas et al. (2007) cloned and characterized the mRNA expression of *hif-1α* and *hif-2α* in *Danio rerio* during development. Shen et al. (2010) completed the cloning of *hif-1α* and *hif-2α* from *Megalobrama amblycephala* and examined their expression in various tissues of adult fish at different embryonic stages, and after acute hypoxia stress. Zhang et al. (2014) cloned the *hif-2α* of *Gymnocypris przewalskii* and investigated the interaction between the oxygen-dependent degradation domain (ODD domain) and proline hydroxylase 3 (PHD3) of the *HIF-2α*. Yang et al. (2022) completed cloning *hif-1α*, *hif-2α*, and *hif-3α* genes in *Phoxinus lagowskii* and investigated their molecular characteristics and expression patterns under hypoxia stress. The *hif*-2α (hypoxia-inducible factor 2 alpha) gene is an important regulator of the cellular response to hypoxia, which plays a crucial role in maintaining oxygen homeostasis in various organisms.

Cobia (*Rachycentron canadum*) is a marine fish species often exposed to hypoxic conditions in its natural environment. This makes it an interesting model for studying the molecular mechanisms underlying hypoxia tolerance. It is a large pelagic migratory fish mainly distributed in the Pacific, Atlantic, Indian Oceans, and other tropical waters (Wang et al., 2021). It has become a critical species for deep-sea aquaculture in the southern coastal areas of China because of its fast growth rate, strong disease resistance, delicate flesh, rich nutrition, and tasty meat (Wang et al., 2021a, b). In recent years, due to natural and anthropogenic factors, the sea area where cobia is cultured is prone to hypoxia, which harms the healthy culture of cobia (Wang et al., 2020; Guo et al., 2020; Wang et al., 2021a, b; Huang et al., 2022). As a critical pathway in hypoxia stress, the HIF-1 signaling pathway has a role in maintaining oxygen transport, angiogenesis, and erythropoiesis. Thus, studying hypoxia-related genes is essential for understanding the mechanisms of hypoxia regulation in fish (Zhang et al., 2017). Huang et al. (2022) have cloned the *hif-1α* in cobia and studied the role of *hif-1α* and its downstream genes *epo* and *vegf*. However, *hif-2α* has not yet been studied. Therefore, this study cloned the full-length sequence of the *hif-2α* in cobia. Real-time fluorescence quantitative PCR (qRT-PCR) was used to detect the expression distribution of *hif-2α* in different tissues, as well as the expression in the liver and gill tissues under hypoxia stress, which will provide a theoretical reference for analyzing the physiological function and regulation mode of *hif-2α* in cobia during hypoxia stress.

Materials and Methods

Experimental materials: The experimental fish were obtained from juveniles bred by the fish seed engineering and breeding team of the Fisheries College of Guangdong Ocean University, Donghai

Table 1. *Hif-2α* gene cloning and qRT-PCR Primers of cobia.

Primer name	Sequence (5'-3')	Application
hif-2α-F1	CTGAGCACCGAGCGAGATTT	Fragment PCR
hif-2α-R1	GCTCTTCTGGGGAAAGGCT	Fragment PCR
3'-F1	CCCACGCACAGGGTCATACAT	RACE
3'-F2	GCAGAAACAGAGGTCCGTTGA	RACE
5'-R1	GCTGGGAGTTGCGGCTGTTAT	RACE
5'-R2	CCTCTCGTCGCAGTAGGTGAACTT	RACE
Long primer	TAATACGACTCACTATAGGGCAAGCAGTG	RACE
Short primer	CTAATACGACTCACTATAGGGC	RACE
qhif-2α-F	ACGCCTTGGACTCAGACAGTGT	qRT-PCR
qhif-2α-R	AGCCTCCGTTCTTAGCCAGCAT	qRT-PCR
β-actin-F	AGGGAAATTGTGCGTGAC	qRT-PCR
β-actin-R	AGGCAGCTCGTAGCTCTT	qRT-PCR

Island, China. Two hundred and ten cobia (body weight: 50.44±2.78 g) of good size, vigor, and fitness were selected and kept in the culture tank for one week to adapt to the new environment before conducting the hypoxia stress experiment. The water temperature was 29±1°C, salinity 28.8±0.5 ppt, and total ammonia nitrogen 0.17±0.03 mg/L, and feeding at 8:00 and 16:00 each day.

Experimental design and sample collection: The experimental design referred to a previous study in the laboratory (Wang et al., 2021a, b). Juvenile cobia were divided into the hypoxia and control groups, with three replicate tanks and 35 fish in each tank. The DO concentration in the hypoxia group was 3.15±0.21 mg/L (Wang et al., 2021a, b). The water DO was reduced by covering it with plastic film and adjusting the amount of aeration and the magnitude of the water flow rate. DO in the water column was measured using the iodometric method (GB7489-87). The control group was not treated and was aerated normally with a DO concentration of 6.18±0.23 mg/L. During the experiment, feeding was done twice daily, and feces and residual bait were cleaned promptly.

At the end of the temporary period, healthy cobia were selected from the control group, anesthetized with MS-222 (100 mg/L), and dissected into nine tissues, including liver, intestine, gill, kidney, muscle, spleen, heart, stomach, and brain for tissue distribution assay by qRT-PCR. Furthermore, samples were taken at 1, 7, 14, and 28 days. Tissues such as liver and gill tissues were dissected, and all samples were snap-frozen in liquid nitrogen and then transferred to -80°C for storage.

Total RNA extraction and cDNA first-strand synthesis: Total RNA was extracted using the TransZol method by grinding the cobia tissues with liquid nitrogen. The concentration and purity were determined using an ultra-micro nucleic acid protein assay, and its integrity was checked by agarose gel electrophoresis. Refer to the instructions of the reverse transcription kit for cDNA first-strand synthesis. Synthesize cDNA according to the instructions of the SMARTer ® RACE5'/3' Kit and store at -20°C.

***hif-2α* gene cloning:** The CDS sequence of the *hif-2α* was obtained using the previous transcriptome data from the liver of cobia in our group. After further validation by Blast comparison, specific primers, *hif-2α* F1 and *hif-2α* R1, were designed using Primer Premier 5.0 (Table 1). Amplified according to the reaction conditions of 94°C for 5 min; 94°C for 30 s, 62°C/64°C for 30 s, 72°C for 80 s, for a total of 35 cycles, and 72°C for 10 min. The products were detected by 1.5% agarose gel electrophoresis. The target bands were purified and recovered, then ligated into the PMD18-T vector and transformed into DH-5α receptor cells. Three monoclonal colonies were formed, selected, and sent to Shanghai Biotechnology for sequencing. The sequenced fragments were then used to design two specific primers, 5'RACE-R1/R2 and 3'RACE-F1/F2 (Table 1), to obtain the 5' and 3'

end sequences of the *hif-2α* by nested PCR.

Bioinformatics analysis of *hif-2α*: For the gene sequencing results, sequence splicing was performed using DNAMAN software to obtain the full-length cDNA sequence of the *hif-2α*. Open reading frame (ORF) and amino acid sequence analysis of HIF-2α was performed by the NCBI database (https://www.ncbi.nlm.nih.gov/orffinder/), TMHMM Server 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) for transmembrane structure analysis, SignalP-5.0 (http://www.cbs.dtu.dk/services/ SignalP/) for signal peptide prediction, and SMART (http://smart.embl-heidelberg.de/) for protein structure and function prediction. The homologous amino acid sequences of HIF-2α from other species were downloaded from the NCBI database. Then, multiple alignment analyses of the sequences were performed using DNAMAN software, and the Neighbor-joining (NJ) method of MEGA 6.0 software was used to construct the phylogenetic tree.

Tissue expression of *hif-2α* and the expression of its in liver and gill tissues after hypoxia stress: Using the sequence of the obtained *hif-2α*, qRT-PCR primers *qhif-2α-F* and *qhif-2α-R* were designed. *β-actin* was used as an internal reference gene for cobia (Table 1). qRT-PCR experimental procedures were performed according to the instructions of the fluorescence quantification kit, using an ABI 7500 real-time fluorescence quantification amplifier (Light cycler 96). The distribution of *hif-2α* expression in 2 tissues of cobia, including the liver and gill, and the expression of *hif-2α* in the liver and gill after hypoxia stress were detected. The qRT-PCR amplification system was 10 μL: 94°C for 30 s, 94°C for 5 s, 60°C for 15 s, 72°C for 10 s, and 40 cycles were performed. Three biological and technical replicates were set for samples and internal reference, and relative expression was calculated using the $2^{-\Delta\Delta CT}$ method. Data were analyzed using SPSS 21.0, $P<0.05$ for significant differences and $P<0.01$ for highly significant differences, and GraphPad Prism 8.0 for graphing. Values are expressed as mean ± standard deviation (SD).

Results

Full-length cDNA sequence cloning and amino acid sequence analysis of *hif-2α*: The cDNA sequence of *hif-2α* was submitted to NCBI GenBank (accession number: OM955932). The result shows a total length of 4021 bp, including the open reading frame (ORF) of 2634 bp, 5' non-coding region 285 bp, and 3' non-coding region 1102 bp, encoding a total of 877 amino acids. Predictions using TMHMM Server 2.0 and SignalP-5.0 did not reveal transmembrane structural domains or signal peptides. Structure-function domain predictions showed that *hif*-2α has an HLH (Helix-loop-helix) structural domain (amino acids 20-75), a PAS (PER-ARNT-SIM) structural domain (amino acids 91-157 and 237-303), and a PAC (PAS Associated C-terminal) structural domain (amino acids 309-352) (Fig. 1).

Amino acid homologous sequence alignment and phylogenetic analysis of *hif-2α*: The results of multiple amino acid sequence alignment showed that the HIF-2α amino acid sequences of cobia had the highest sequence identity with *Seriola dumerili*, *Toxotes jaculatrix*, and *Seriola lalandi dorsalis*, with 92.87, 92.83, and 92.74%, respectively. 92.50, 91.57, and 89.31% for *Xiphias gladius*, *Echeneis naucrate*s, and *Siniperca cheats*. The sequence identity with *Homo sapiens* and *Mus musculus* was lower at 57.02 and 55.99%, respectively (Table 2, Fig. 2).

The phylogenetic tree shows that the cobia forms a branch with other bony fishes. The closest relatives are *E. naucrates*, which form a small branch, and *D. rerio*, which is more distantly related. In addition, mammals, amphibians, and other higher chordates cluster into a single branch and are more distantly related to the cobia (Fig. 3).

Tissue expression analysis of *hif-2α*: The RT-qPCR was used to examine the tissue distribution and expression levels of *hif-2α* under normal conditions. The results showed that the *hif-2α* was expressed in all nine tissues of juvenile cobia. The liver has the highest expression compared to stomach tissue, followed by the gill, and lower expression in the brain, heart, somatic kidney, muscle, spleen, intestine, and stomach (Fig. 4).

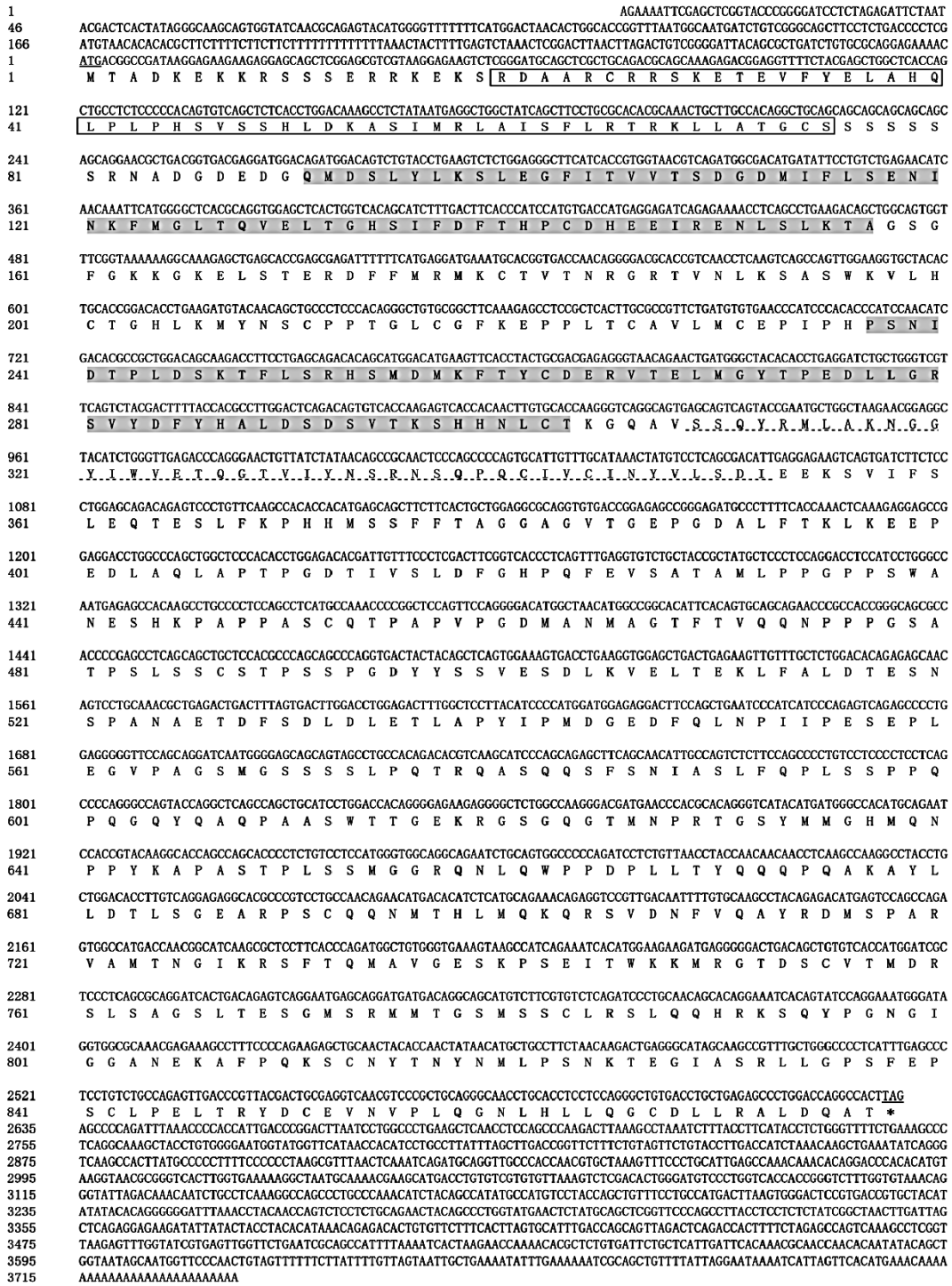

Figure 1. Complete sequence of HIF-2α cDNA and amino acid sequence analysis of cobia (Note: The single underscore is the start codon (ATG), the double underscore is the stop codon (TAG), the box is the HLH domain, the gray is the PAS domain, and the dotted is the PAC domain).

Expression of *hif-2α* in the liver and gill tissues after hypoxia stress: The expression of *hif-2α* in cobia at different time points after hypoxia stress is shown in Figure 5. The results showed that in the liver, the *hif-2α* was not significantly different at 1 and 7 d after stress, was significantly higher than the control at 14 d ($P<0.05$), and was highly significant at 28 d ($P<0.01$) (Fig. 5A). In the gills, *hif-2α* expression was not significantly different at 1 d post-stress, significantly lower at 7 and 28 d ($P<0.05$), and most different at 14 d compared to the control ($P<0.01$) (Fig. 5B).

Table 2. HIF-2α polypeptide sequence identity of cobia with 16 other species

Matched species	Accession no.	Identity
Seriola dumerili	XP_022603872.1	92.87%
Toxotes jaculatrix	XP_040905433.1	92.83%
Seriola lalandi dorsalis	XP_023250442.1	92.74%
Xiphias gladius	XP_039995546.1	92.50%
Echeneis naucrates	XP_029376549.1	91.57%
Siniperca chuatsi	XP_044070053.1	89.31%
Chelmon rostratus	XP_041802908.1	88.54%
Hippoglossus stenolepis	XP_035027600.1	87.37%
Morone saxatilis	XP_035519566.1	87.29%
Anabas testudineus	XP_026209977.1	87.19%
Danio rerio	NP_001034895.2	66.78%
Homo sapiens	XP_011531000.1	57.02%
Gallus gallus	XP_015139105.2	56.31%
Rana temporaria	XP_040207494.1	56.10%
Xenopus tropicalis	NP_001005647.1	56.08%
Mus musculus	NP_034267.3	55.99%

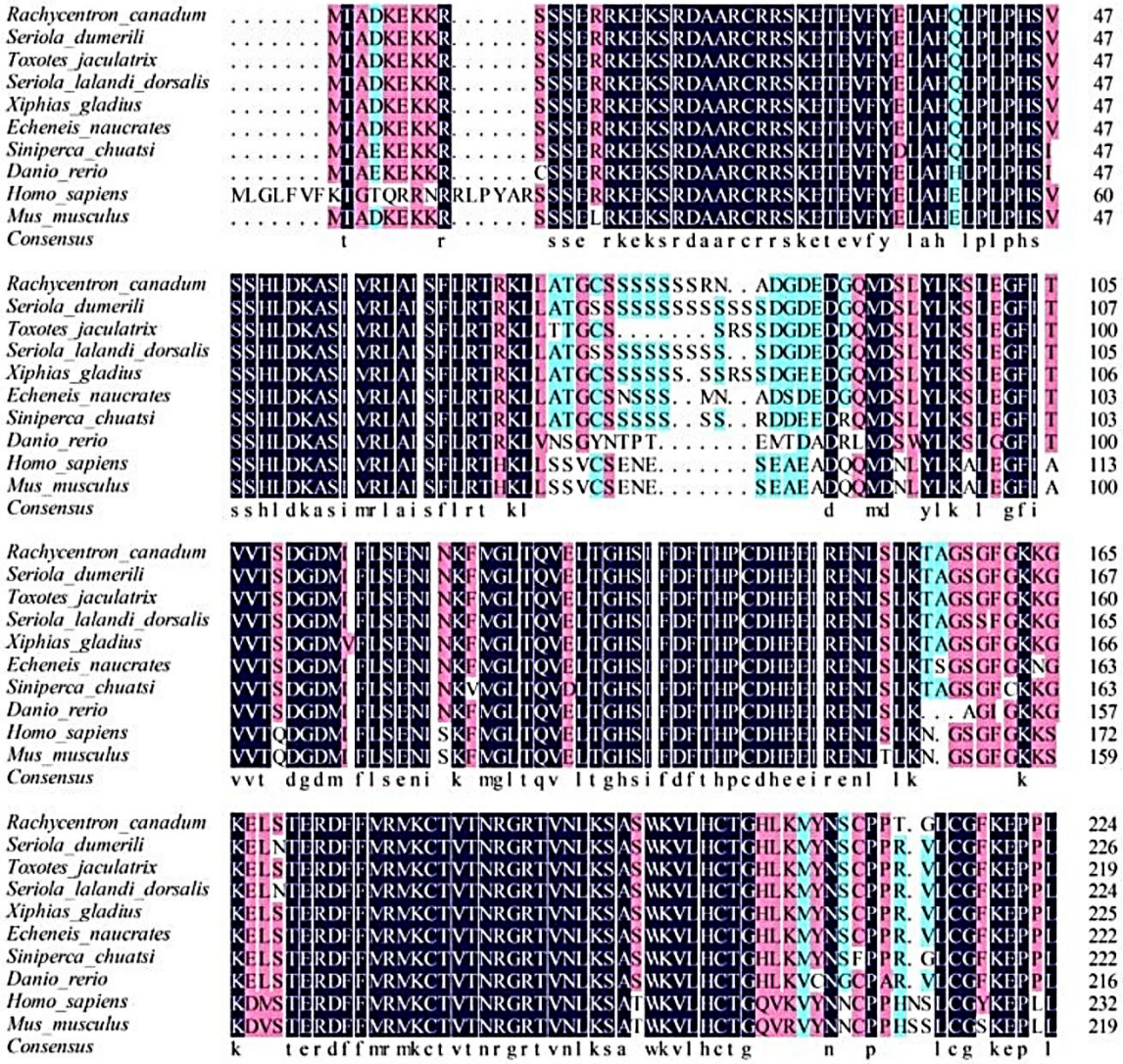

Figure 2. Analysis of HIF-2α amino acid sequences of cobia by multiple sequence alignment.

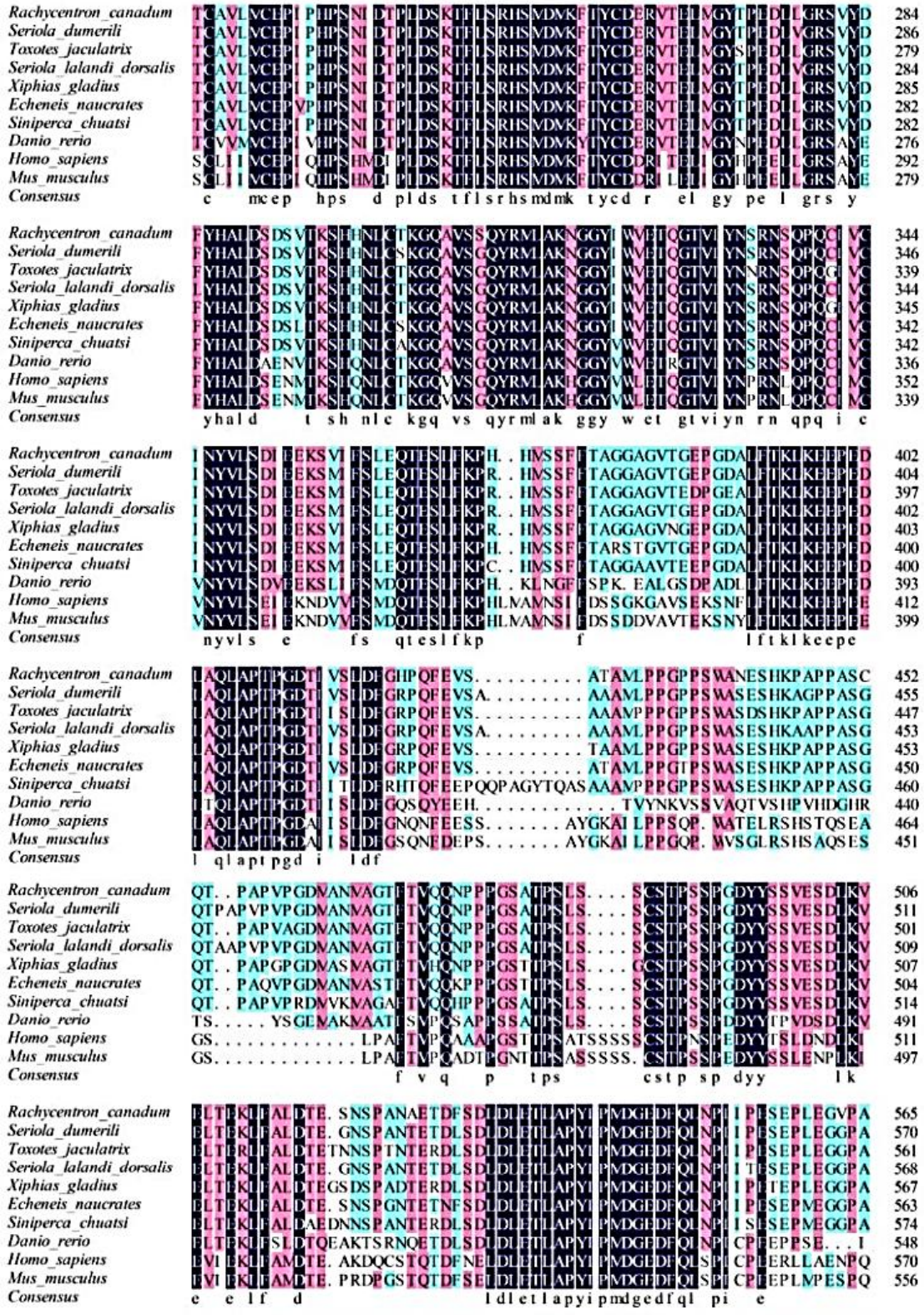

Figure 2. Continued.

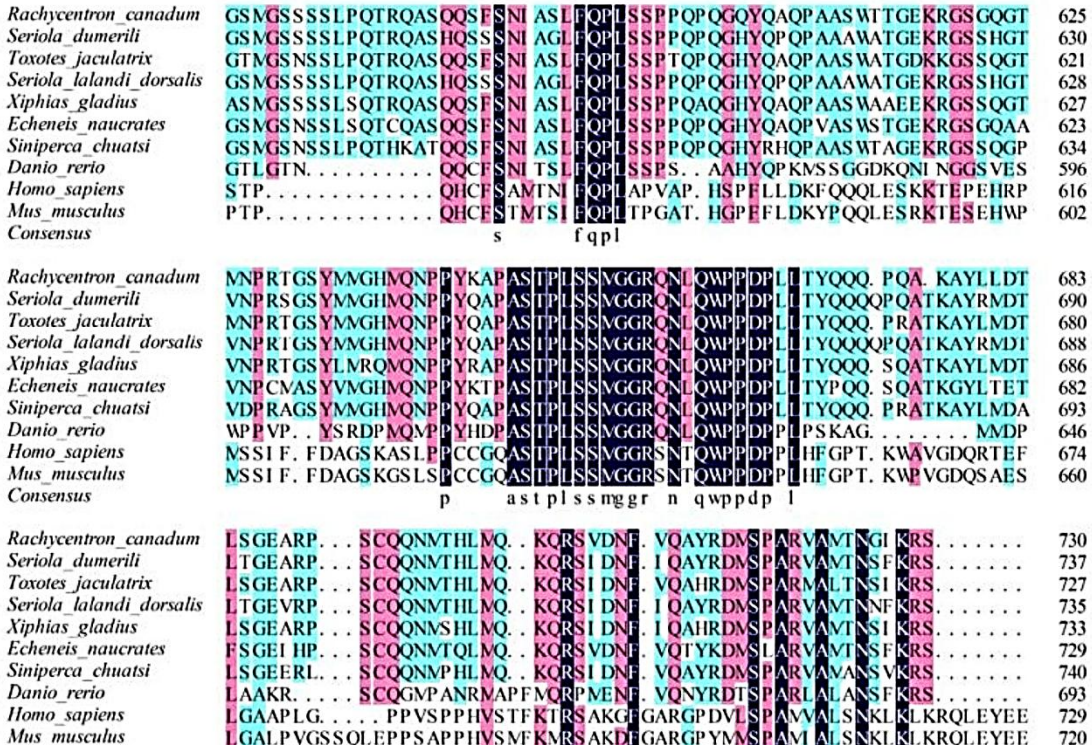

Figure 2. Continued.

Discussions

Hypoxia, or a low oxygen environment, can significantly affect the physiology and behavior of aquatic animals. In response to hypoxia, animals activate a range of physiological and molecular responses to maintain oxygen homeostasis and prevent tissue damage. One of the key molecular responses to hypoxia is the upregulation of hypoxia-inducible factors (HIFs), which are transcription factors that regulate the expression of genes involved in oxygen homeostasis, energy metabolism, angiogenesis, and erythropoiesis. In fish, the HIF-α subunit is typically stabilized under hypoxia and can activate downstream target genes.

Hypoxia is a common environmental stressor that affects fish survival and growth. The hypoxia-inducible factor (HIF) is a transcription factor that plays a critical role in the adaptive response to hypoxia by regulating the expression of genes involved in oxygen homeostasis. HIF-2α, a subunit of the HIF complex, has been identified as an important regulator of the hypoxic response in fish. The HIF-1 signaling pathway is critical for cells to generate hypoxic responses, and HIFs play an essential role in the hypoxia signaling pathway (Xiao, 2015; Lin et al., 2020).

Cloning and expression analysis of the *hif*-2α gene in fish under hypoxia stress have been reported in several studies. For example, Xu et al. (2019) cloned and characterized the *hif-2α* gene from the Chinese sturgeon (*Acipenser sinensis*) and found that its expression was significantly upregulated in response to hypoxia. Similarly, Huang et al. (2017) cloned and analyzed the hif-2α gene in the yellow catfish (*Pelteobagrus fulvidraco*) and observed a significant increase in its expression under hypoxic conditions. In addition to these studies, other researchers have also investigated the expression patterns of *hif*-2α in different fish species under hypoxia stress. For instance, *Wang et al. (2017)* studied the expression of *hif*-2α in the largemouth bass (*Micropterus salmoides*) and observed a significant increase in its expression in response to hypoxia. Similarly, Li et al. (2017) analyzed the expression of *hif*-2α in the grass carp (*Ctenopharyngodon idellus*) and found that its

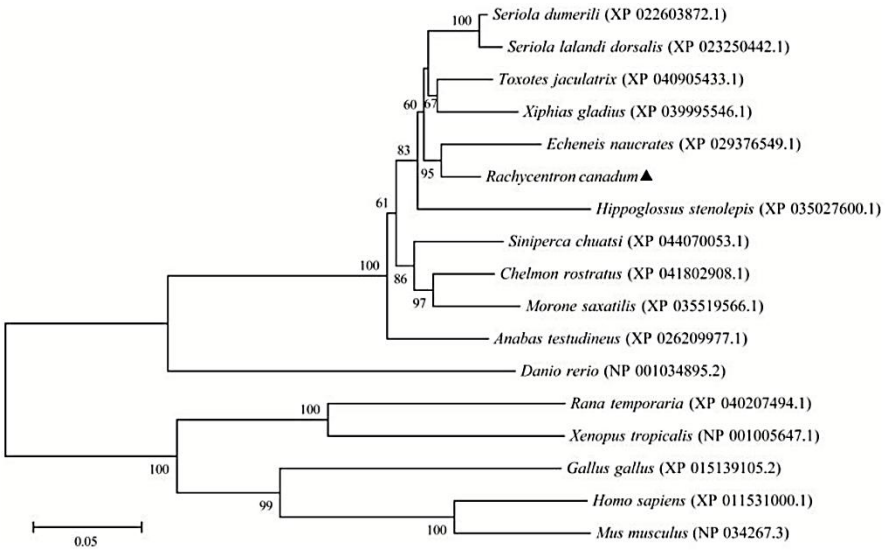

Figure 3. Phylogenetic tree of HIF-2α amino acid sequences of different species based on the Neighbor-Joining (NJ tree) method.

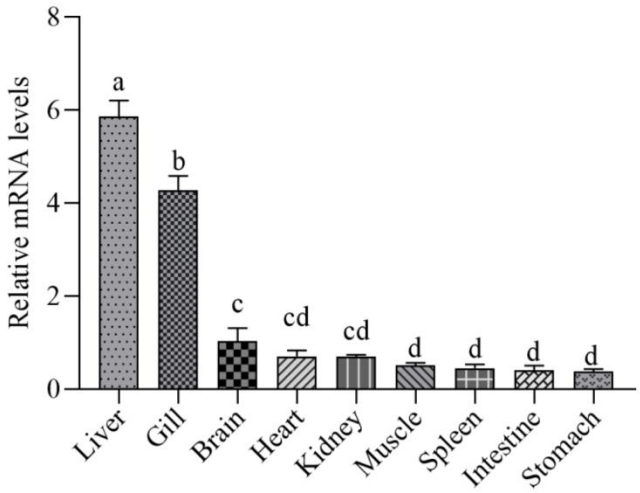

Figure 4. The expression levels of hif-2α mRNA in different tissues of cobia.

expression was significantly upregulated under hypoxic conditions.

In this study, the full-length sequence of the *hif-2α* of cobia was cloned for the first time by the RACE technique, which was 4021 bp in length and encoded 877 amino acids, and showed 92.87% identity with high body amber. Cobia HIF-2α contains three functional structural domains: the bHLH structural domain, the PAS structural domain, and the PAC structural domain, which are similar to those of HIF-1α (Huang et al., 2022) are similar to those identified so far in blunt snout bream (Shen et al., 2010), *Schizothorax prenanti*, *Gymnocypris doubla*, and other species with similar structural domains of HIF-2α homologs (Jiang et al., 2015). Studies have reported that the N-terminal bHLH and PAS structural domains play essential roles in subunit dimerization and DNA binding functions (Bruick, 2003; Shen et al., 2010). Although there are some sequence differences between the cobia *hif-2α* and other species, they have similar functional and structural domains, suggesting that the cobia *hif-2α* is relatively conserved in its functional sites and structural domains across species evolution (Bruick, 2003; Shen et al., 2010). In multiple sequence comparisons, the amino acid sequence of cobia HIF-2α was more similar to other bony fishes and less similar to amphibians and mammals. Combined with the results of evolutionary tree analysis, it was more closely related to other bony fishes and more distant from amphibians and mammals under the pattern of species evolutionary process. This suggests that the *hif-2α* obtained by our cloning belongs to the fish *hif-2α* homolog.

In species such as blunt snout bream (Shen et al., 2010) and *P. lagowskii* (Yang et al., 2022), the *hif-2α* was expressed in the organism's primary tissues under normoxic conditions, similar to our findings. The *hif-2α* of cobia was expressed in 9 tissues, including the liver and gill. In addition, we also observed some differences in the expression pattern of the *hif-2α* in different fish tissues: high expression in the gill,

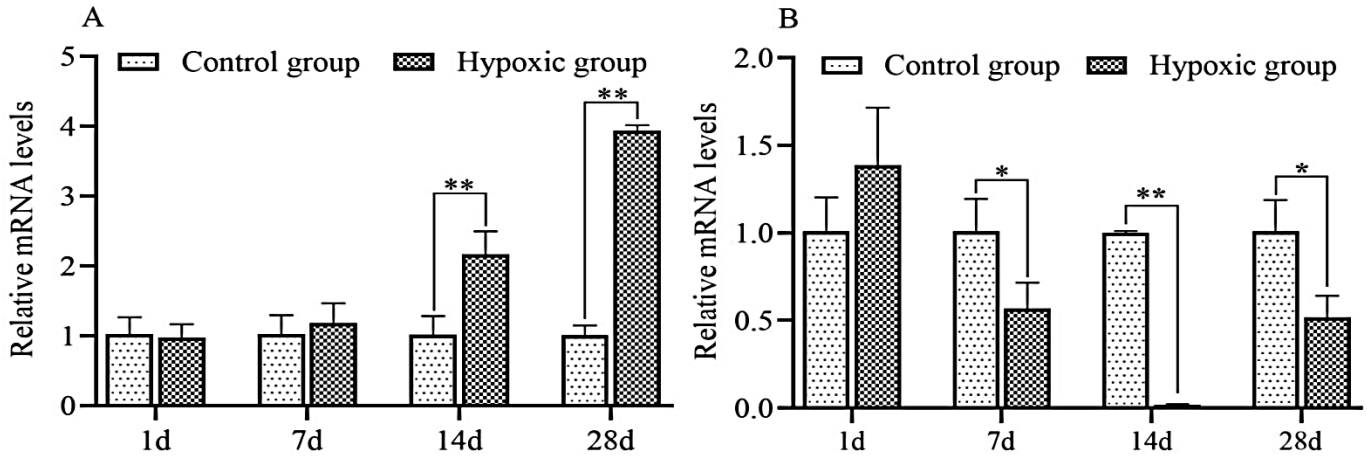

Figure 2. Expression levels of *hif-2α* mRNA (A): liver and (B): gill tissues of cobia after hypoxia stress (Note: *shows a significant difference (P<0.05), **shows a highly significant difference (P<0.01)

ovary, and brain of *D. rerio* (Rojas et al., 2007), highest expression in the spleen and heart, and lowest in muscle and intestine of *P. lagowskii*, high expression in liver and gill tissues and lower expression in other tissues of cobia. Jiang et al. (2015) found that the *hif-2α* was expressed in four tissues, namely gill, brain, skin, and spleen, in *S. prenanti* and *G. doubla*, and the expression in *G. doubla* was lower than in *S. prenanti*. The above results indicated that the tissue expression pattern of the *hif-2α* was species and tissue-specific, and its high expression in the liver and gill of juvenile cobia suggested that the *hif-2α* might have an essential regulatory role in oxidative stress in these two tissues. The liver, an essential fish organ in response to oxidative stress, plays a critical role in nutrient metabolism, detoxification, and host defense (Liu et al., 2015). As the most direct organ of oxygen acquisition in fish, the gills are the first to sense the effects of hypoxia on the organism and make rapid coping strategies (Chen et al., 2017; Abdel-Tawwab et al., 2019).

The present study characterised HIF-2α expression patterns under hypoxia stress. The study found that HIF-2α mRNA expression was significantly upregulated in cobia liver tissues after exposure to hypoxia at days 7, 14, and 28. The HIF-2α mRNA expression was upregulated in the cobia gill after exposure to hypoxia at day 1, but significantly downregulated at days 7, 14, and 28. Studying *hif-2α* expression in cobia's liver and gill tissues under hypoxia stress has theoretical implications for analyzing hypoxic response mechanisms in cobia. It has been reported that the different levels of *hifs* mRNA expression in different tissues of fish may be related to the sensitivity of sensing oxygen, the tissues subjected to hypoxia, and the degree of hypoxia (Xu et al., 2019; Wu et al., 2019). The α-subunit of HIFs was rapidly activated after hypoxia occurred and later binds to the hypoxia response element (HRE) of the corresponding downstream target gene, which activates the expression of downstream genes in response to hypoxia (Xiao, 2015; Zhang et al., 2020). In studies of hypoxia stress in teleost fish such as *Lateolabrax maculatus* (Zhang et al., 2020), *M. amblycephala* (Shen et al., 2010), *Myxocyprinus asiaticus* (Chen et al., 2012), and *Sebastes schlegeli* (Mu et al., 2015), the expression of *hif-2α* in the liver significantly increased after hypoxia. Compared to the results of our previous studies, the expression of *epo* and *vegf*, the downstream genes of *hifs,* increased significantly in the liver after prolonged hypoxia stress (14 and 28 days) (Huang et al., 2022), suggesting that the *hif*-2α gene may be activated in the liver of cobia together with *epo* and *vegf* genes in response to hypoxic stress. In this study, the expression of *hif-2α* in the liver of cobia did not significantly change at 1 and 7 days. After hypoxia stress, it was significantly higher than the control level at 14 and 28 days. Similar to *Micropogonias undulatus* (Rahman and Thomas, 2007), the expression of the *hif-2α* was not changed significantly after 3 days of hypoxia stress and increased dramatically after 7 and 21 days of stress.

These results suggest that *hif-2α* may not be involved in the early stages of hypoxia adaptation, but instead functions in long-term hypoxia stress.

It has been reported that *S. prenanti* adapts to prolonged low-oxygen conditions on the plateau by down-regulating the expression of *hif-α* isoforms (Jiang et al., 2025). In addition, Tibetan populations may adapt to prolonged plateau hypoxia by down-regulating the expression of *hif-1α/2α, epo*, possibly through the lower expression of these genes, resulting in lower hemoglobin counts and faster blood flow to maintain oxygen homeostasis in the body (Petousi et al., 2014). In the present study, the expression of *hif-2α* in the gill tissue was not changed significantly at 1 d and decreased significantly at 7, 14, and 28 d. It was assumed that the regulatory mechanism of *hif-2α* in response to prolonged hypoxia in cobia may be similar to that in *S. prenanti* and Tibetan populations or that prolonged hypoxia stress caused severe damage to gill tissue (Chen et al., 2017), resulting in lower than average expression of *hif-2α*. In addition, *hif-2α* was in contrast to the results of the previous study on the high expression of the *hif-1α* gene in gills after hypoxia (Huang et al., 2022), suggesting that the *hif-1α* and *hif-2α* of cobia have different activation domains and thus play different regulatory functions in response to hypoxia stress (Wu et al., 2019). In summary, the regulatory mechanisms of *hif-2α* in hypoxia response are influenced by the duration and intensity of stress, and there are species and tissue differences in their expression patterns.

Conclusion

This study successfully cloned the *hif-2α* of cobia. The study suggests that hif-2α plays a crucial role in the hypoxic response of fish and its expression is upregulated in the liver but downregulated in gill under hypoxia stress. It also suggested that the HIF-2α pathway was activated in cobia under hypoxia stress. The cloning and expression analysis of the hif-2α gene in cobia under hypoxia stress provide valuable insights into the molecular mechanisms underlying the hypoxic response in fish.

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