



Contribution of HIF-1 α to Heat Shock Response by Transcriptional Regulation of HSF1/HSP70 Signaling Pathway in Pacific Oyster, *Crassostrea gigas*

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Abstract

Ocean temperature rising drastically threatens the adaptation and survival of marine organisms, causing serious ecological impacts and economic losses. It is crucial to understand the adaptive mechanisms of marine organisms in response to high temperature. In this study, a novel regulatory mechanism that is mediated by hypoxia-inducible factor-1 α (HIF-1 α) was revealed in Pacific oyster (*Crassostrea gigas*) in response to heat stress. We identified a total of six HIF-1 α genes in the *C. gigas* genome, of which *HIF-1 α* and *HIF-1 α -like5* were highly induced under heat stress. We found that the *HIF-1 α* and *HIF-1 α -like5* genes played critical roles in the heat shock response (HSR) through upregulating the expression of heat shock protein (HSP). Knocking down of HIF-1 α via RNA interference (RNAi) inhibited the expression of heat shock factor 1 (HSF1) and HSP70 genes in *C. gigas* under heat stress. Both *HIF-1 α* and *HIF-1 α -like5* promoted the transcriptional activity of HSF1 by binding to hypoxia response elements (HREs) within the promoter region. Furthermore, the survival of *C. gigas* under heat stress was significantly decreased after knocking down of HIF-1 α . This work for the first time revealed the involvement of HIF-1 α /HSF1/HSP70 pathway in response to heat stress in the oyster and provided an insight into adaptive mechanism of bivalves in the face of ocean warming.

Keywords Thermal tolerance · Pacific oyster · Heat shock response · HIF-1 α

Introduction

Global climate change has driven environmental changes dramatically, including global temperature rise, shrinking ice sheets, and ocean warming. Global ocean warming is a critical indicator of the climate system. Surface temperature of the ocean has hit record high in 2020, and a continued increasing has been predicted (Cheng et al. 2021). The healthy and sustainable development of coastal ecosystem under ocean temperature rising is of substantial concern (McLeod et al. 2019; Solan and Whiteley 2016). Ocean temperature rising that linked to global warming was predicated

to threaten the survival of marine animals, especially those distribute in coastal zones (Chen et al. 2021; Khan et al. 2020; Petes et al. 2007; Pinsky et al. 2019).

Marine bivalves play unique and essential roles in marine ecosystem with their worldwide distribution, ecological significance, economic benefits, and food resources (Guo et al. 2008; McLeod et al. 2019; Strehse and Maser 2020; Wijnsman et al. 2019). However, the sessile bivalves are vulnerable to high temperature particularly during tidal fluctuations in summer since they are not able to escape from the habitats (Chapman 2006; Freitas et al. 2021). High temperature caused damages on the organs of marine bivalves, posing cascading effects on various physiological processes including immunity, metabolism, growth, reproduction, and development (Li et al. 2009, 2007; Mosca et al. 2013; Patrick et al. 2006; Zippay and Helmuth 2012). It is also worth noting that the heat stress is frequently accompanied by hypoxia stress, which together aggravates the damage to the marine organisms (Hu et al. 2022; Huo et al. 2019; McArley et al. 2020; Vaquer-Sunyer and Duarte 2011). Consequently, it is of great significance to discover the molecular mechanisms

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underlying the response of marine bivalves to high temperature, which will provide insights into understanding the adaptation of marine organisms to ocean warming.

The heat shock response (HSR) is an evolutionarily conserved cellular process, and it is particularly essential for the thermal tolerance of organisms under heat stress (Deka et al. 2016; Feder and Hofmann 1999; Somero 2020; Tomanek 2010). Although the HSR was firstly discovered in fruit flies under heat shock (Ritossa 1962), it was found to be a crucial mechanism to protect cells from various stimuli including hypoxia (Baird et al. 2006; Klumpen et al. 2017; Luo et al. 2021; Michaud et al. 2011). It was also reported that the heat shock transcription factor 1 (HSF1) was induced under hypoxia (Baird et al. 2006; Benjamin et al. 1990; Giaccia et al. 1992). The hypoxia-inducible factor-1 α (HIF-1 α) was an important transcription factor of hypoxia response (Semenza 2007). Previous studies suggested that it was also an evolutionarily conserved and a necessary component of heat acclimation (Treinin et al. 2003). HIF-1 α regulated gene transcription by binding to the hypoxia response elements (HREs) within the promoter regions of target genes (Semenza 2007). Genes like HSP70 and HSF1 that involved in HSR were regulated via the HIF-1 α pathway under hypoxic condition (Luo et al. 2021). Although it has been reported that HIF-1 α activated the HSR via regulation of HSF1 (Agarwal and Ganesh 2020), the specific regulatory relationship between the two transcription factors is poorly understood in marine bivalves under heat stress.

The Pacific oyster (*Crassostrea gigas*) is widely distributed worldwide with important ecological and economic values which serve as an excellent model to study the adaptation of marine invertebrates to coastal environments (Dong et al. 2022; Guo 2009; Zhang et al. 2012). In this study, we found that the HIF-1 α genes were induced in *C. gigas* in response to heat stress, resulting in the activation of HSR and contributing to thermal tolerance of *C. gigas* under heat stress. We for the first time revealed the role of HIF-1 α /HSF1 pathway in oyster in response to high-temperature stress. Our findings suggested that HIF-1 could serve as a critical molecular integrator associated with thermal tolerance in a marine bivalve.

Materials and Methods

Gene Identification

The homologous sequence alignment was performed to identify the full set of the HIF-1 α genes in *C. gigas*. We retrieved the HIF-1 α protein sequences of representative species from NCBI database (<https://www.ncbi.nlm.nih.gov/>), including vertebrates (*Homo sapiens*, *Mus musculus*, *Meleagris gallopavo*, *Gallus gallus*, *Anolis carolinensis*, *Chrysemys picta bellii*, *Xenopus laevis*, *Xenopus*

tropicalis, *Danio rerio*, *Pangasianodon hypophthalmus*, *Branchiostoma belcheri*) and invertebrates (*Ciona intestinalis*, *Acanthaster planci*, *Strongylocentrotus purpuratus*, *Mizuhopecten yessoensis*, *Crassostrea virginica*, *Bombyx mori*, *Caenorhabditis elegans*, *Exaiptasia diaphana*) (Supplementary Table 1). The downloaded sequences were then searched against the *C. gigas* genome (GenBank: GCA_902806645.1) using BLAST program with the E-value of 1E-10. The obtained sequences were finally confirmed by protein BLAST (BLASTP) (Altschul et al. 1997) against NCBI non-redundant (Nr) protein sequence database and manually curated using the Conserved Domain Search Service (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). The gene location in the chromosome, the number of amino acids, and the base number of mRNA and UPR of the identified gene members were investigated according to the genome of *C. gigas* (GenBank: GCA_902806645.1).

Phylogenetic Analysis

The phylogenetic analysis of HIF-1 α was performed using the protein sequences of *C. gigas* and representative species (*H. sapiens*, *M. musculus*, *M. gallopavo*, *G. gallus*, *C. picta*, *P. sinensis*, *D. rerio*, *X. laevis*, *X. tropicalis*, *P. maximus*, *M. yessoensis*, and *C. virginica*). The aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIF-1 β) protein sequences of those species were also included in order to clearly distinguish the HIF-1 α members. The phylogenetic tree was constructed using neighbor-joining (NJ) approach in MEGA7 (Kumar et al. 2016) with 1000 bootstraps.

Plasmid Construction

The AnimalTFDB 3.0 (<http://bioinfo.life.hust.edu.cn/AnimalTFDB#!/>) and JASPAR (<http://jaspar.genereg.net/>) were used to predict the putative binding sites of HIF-1 α on promoter region of HSF1 (LOC105328117). The HSF1 promoter sequences containing different putative binding sites were amplified with specific primers and inserted into the NheI/HindIII site of pGL3-basic vector (Promega, USA) to construct the reporter plasmids (pGL3-HSF1). The open reading frame (ORF) of *HIF-1 α* and *HIF-1 α -like5* was amplified and ligated to the pcDNA3.1(+) expression vector (Invitrogen, USA) to construct expression plasmids, respectively. The recombinant plasmids were constructed using the ClonExpress II One-Step Cloning Kit (Vazyme, Nanjing, China). Plasmids for transfection were prepared using Endo-Free Max Plasmid Kit II (Tiangen, China) according to the manufacturer's instructions. The primers used for plasmid construction were listed in Supplementary Table 2.

Cell Culture, Transfection, and Dual-Luciferase Reporter Assay

The HEK293T cells were cultured in DMEM high-glucose medium (Hyclone, USA) containing 10% FBS (Hyclone, USA) and 1% penicillin/streptomycin in incubator with 5% CO₂ at 37 °C. After 24 h culturing in 24-well plates, the cells were co-transfected with 0.5 µg of reporter plasmids and 0.25 µg of expression plasmids and 0.1 µg of pRL-TK Renilla luciferase plasmids (Promega, USA) using Xfect Transfection Reagent (Takara, Japan) according to the manufacturer's protocol. After 48-h transfection, the Firefly luciferase and Renilla luciferase activities were determined using Dual-Luciferase Reporter Assay System (Promega, USA) according to the manufacturer's instructions on a SYNERGY H1 microplate reader (BioTek, USA).

Long Double-Stranded RNA (dsRNA) Preparation

The *HIF-1α* and *HIF-1α-like5* target cDNA fragment was amplified with primer pairs (Supplementary Table 3), respectively. The cDNA fragment was added T7 promoter by cloning to the pGEM[®]-T vector (Promega, USA). The linearized plasmid templates were used to synthesize dsRNA using T7 RiboMAX[™] Express RNAi System (Promega, USA) according to the manufacturer's instructions. The quality and concentration of dsRNA were assessed by 1.5% agarose gel electrophoresis and Nanodrop 2000 spectrophotometer (Thermo Scientific, USA).

Experiment Animals, RNAi, and Heat Shock Treatment

The healthy 1-year-old Pacific oysters (55 ± 6 mm in shell height) were collected from Weihai, China (July 2020), and transported to laboratory for experiment. The oysters were drilled with a small hole on shell near adductor muscle and were kept in filtered seawater (20 ± 0.5 °C) for 1-week acclimation. During the acclimation period, oysters were fed with algae, and the water was changed daily. After acclimation, the oysters were randomly divided into three groups, the *HIF-1α* dsRNA injection group ($n = 66$), the *HIF-1α-like5* dsRNA injection group ($n = 66$), and the PBS injection group ($n = 66$). The oysters were individually injected with 100 µL of 50 µg/100 µL *HIF-1α* dsRNA, 100 µL of 50 µg/100 µL *HIF-1α-like5* dsRNA, or 100 µL 1 × PBS into the adductor muscle using micro syringe (Tian et al. 2021). The injected oysters were placed into tanks with filtered seawater at 35 ± 0.5 °C (Liu et al. 2019). The gills of six individuals were randomly sampled at 0, 3, 12, and 24 h from each group after injection. The gills were dissected, flash-frozen in liquid nitrogen, and stored at –80 °C freezer until use for qRT-PCR analysis. Dead oysters were counted

and removed from the tank during the experiment. Thirty-six individuals were used in each group.

Gene Expression Analysis

The mRNA expression levels of HIF-1α members and key genes in HSR pathway were determined in *C. gigas* under chronic heat stress (30 °C) as previously reported (Fu et al. 2021). Briefly, the oysters were placed in tanks with a constant flow system, and the water was elevated at a rate of 1 °C/h to 30 °C. Total RNA was extracted from gills with TRIzol[®] reagent (Invitrogen, USA) and used for cDNA synthesis with PrimeScript[™] RT Master Mix Perfect Real-Time Kit (Takara, Japan) according to the manufacturer's introductions. The qRT-PCR was carried in triplicates (two individuals per replicate) on a LightCycler 480 real-time PCR machine (Roche, Switzerland) with a total volume of 10-µL reaction mix using SYBR Green PCR Master Mix (QIAGEN, Germany) with the following conditions: 95 °C for 2 min, 40 cycles at 95 °C for 5 s, and 60 °C for 10 s. The mRNA relative expression level of gene was calculated using the relative 2^{-ΔΔCT} method (Livak and Schmittgen 2001). The EF-1α gene was used as control as previously reported (Liu et al. 2019). All the primers used for qRT-PCR analysis were listed in Supplementary Table 4.

Results

Identification of HIF-1α Genes in *C. gigas* Genome

A total of six HIF-1α homologous genes were identified in the *C. gigas* genome (Table 1). All of the members contain the classic conserved domains of HIF-1α, including basic-helix-loop-helix (bHLH) and Per-Arnt-Sim (PAS). The genes were renamed according to their location in the chromosome except for the *HIF-1α* (Gene ID: 105324565) which has been well annotated in NCBI. The phylogenetic analysis suggested that HIF-1α genes in *C. gigas* were clustered with those from invertebrates, and there was an obvious distinction between the HIF-1α and the ARNT (HIF-1β) clades (Fig. 1).

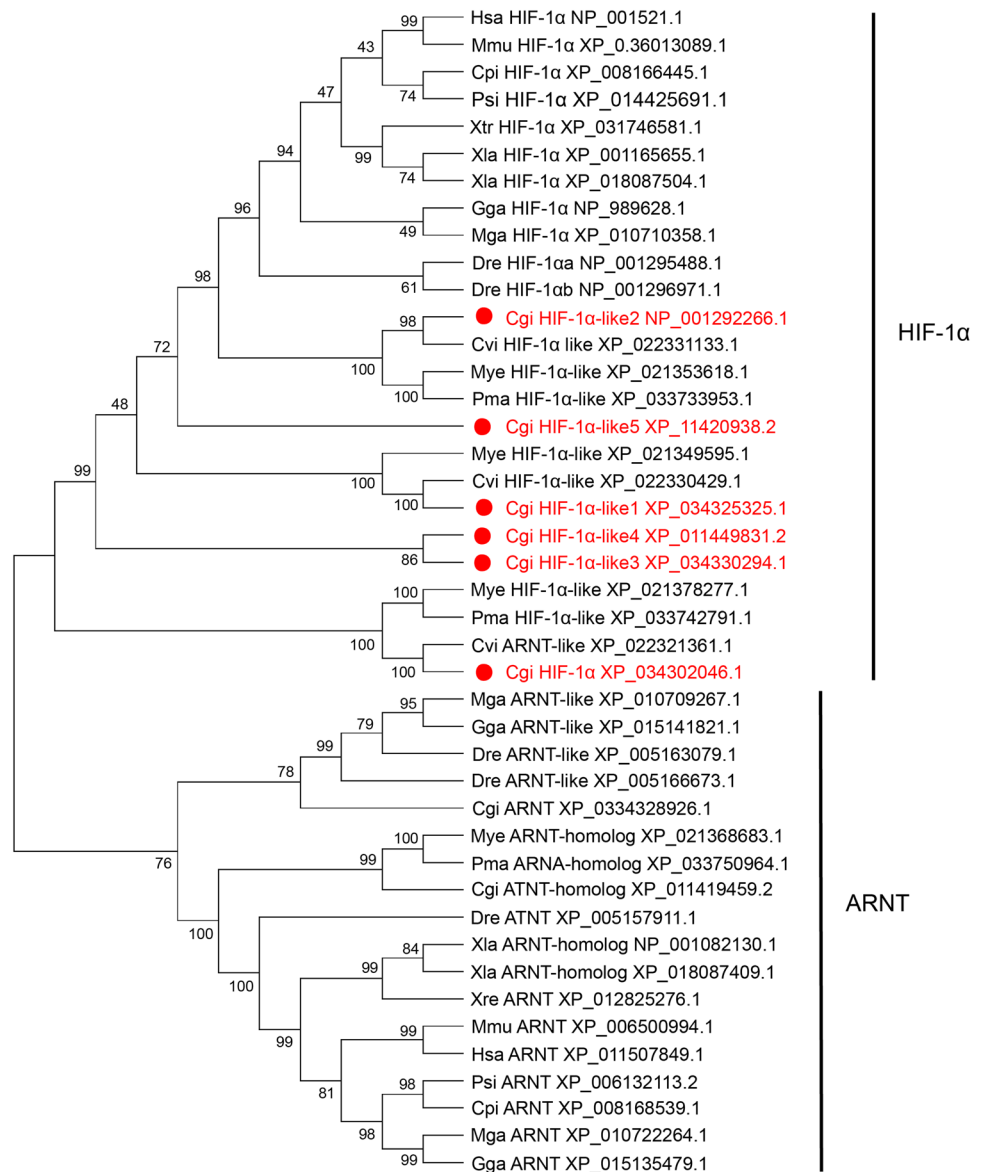
HIF-1α Genes Were Induced in *C. gigas* Under Heat Stress

In order to determine whether the HIF-1α genes were involved in response to heat stress, the mRNA expression levels of HIF-1α members were determined in *C. gigas* under chronic heat stress (30 °C). The results showed that *HIF-1α* and *HIF-1α-like5* were significantly induced in *C. gigas* at 22 h and 34 h post-heat stress ($P < 0.05$) (Fig. 2).

Table 1 Summary of HIF-1 α genes identified in *C. gigas* genome

Gene ID	Gene name	Conserved domain	Amino acid (aa)	mRNA (bp)	3'-UTR (bp)	5'-UTR (bp)	Chromosome location
105343010	<i>HIF-1α-like1</i>	bHLH_PAS, PAS_3, PAS	889	5094	167	2707	3: 53963762–53999400
105339544	<i>HIF-1α-like2</i>	bHLH-PAS_HIF, PAS_3, PAS, HIF-1 super family	698	3182	409	2506	3: 54630077–54659712
105336391	<i>HIF-1α-like3</i>	bHLH_SF super family, PAS_3, PAS	755	2518	75	175	4: 16439666–16451024
105343987	<i>HIF-1α-like4</i>	bHLH-PAS_spineless_like, PAS_3, PAS	814	3495	526	524	5: 27402074–27437089
105323580	<i>HIF-1α-like5</i>	bHLH_SF super family, PAS_3, PAS	701	3648	682	2788	5: 55345664–55372277
105324565	<i>HIF-1α</i>	bHLH_SF super family, PAS_11 super family, PAS	760	6979	121	2404	7: 21284187–21305460

Fig. 1 Phylogenetic analysis of HIF-1 α genes in *C. gigas*. The phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 7 software with 1000 bootstraps. Abbreviations: Hsa, *Homo sapiens*; Mmu, *Mus musculus*; Mga, *Meleagris gallopavo*; Gga, *Gallus gallus*; Cpi, *Chrysemys picta*; Psi, *Pelodiscus sinensis*; Dre, *Danio rerio*; Xla, *Xenopus laevis*; Xtr, *Xenopus tropicalis*; Pma, *Pecten maximus*; Mye, *Mizuhopecten yessoensis*; Cvi, *Crassostrea virginica*; Cgi, *Crassostrea gigas*. The *C. gigas* HIF-1 α were labeled by red spot



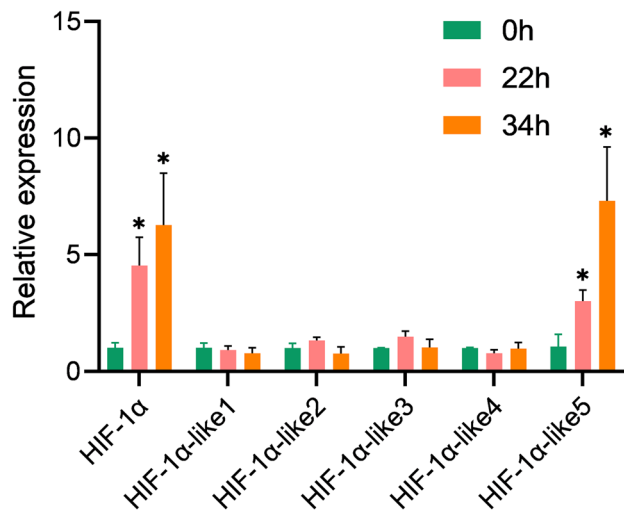


Fig. 2 Relative expression of HIF-1 α genes in *C. gigas* under chronic heat stress. Values were shown as the mean \pm SD ($n=3$). Asterisk indicates significant difference compared with control (0 h) (* $P<0.05$)

Therefore, we focused on these two members for further analyses.

Knocking Down of HIF-1 α Inhibited the Expression of HSF1 and HSP70 Genes in *C. gigas* Under Heat Stress

The expression of HSF1 and HSP70 genes was used to monitor the HSR. Apparently, both of HSF1 and HSP70 were significantly induced in *C. gigas* under chronic heat stress ($P<0.05$) (Fig. 3). In order to confirm the involvement of HIF-1 α in the HSR of *C. gigas* under heat stress, the RNAi treatment through dsRNA injection was used to knock-down the targeted HIF-1 α homologs (Fig. 4). The mRNA expression level of *HIF-1 α* was continuously decreased after *HIF-1 α* dsRNA injection and significantly lower than that in PBS injection group. Notably, injection of *HIF-1 α* dsRNA also significantly decreased the relative expression levels of *HIF-1 α -like5* after 24-h injection ($P<0.05$) (Fig. 4A). Similarly, both mRNA expression levels of *HIF-1 α* and the *HIF-1 α -like5* were significantly decreased after *HIF-1 α -like5* dsRNA injection, while there was no significant difference at 24 h post-injection compared with 0 h and PBS injection group ($P<0.05$) (Fig. 4B). Moreover, the mRNA expression of HSF1 was significantly suppressed at 24 h after injection of *HIF-1 α* dsRNA or *HIF-1 α -like5* dsRNA ($P<0.05$) (Fig. 4C). Although the mRNA expression of HSP70 continued to decrease after dsRNA injection of *HIF-1 α* or *HIF-1 α -like5*, it was significantly higher in *HIF-1 α -like5* dsRNA injection group than that in *HIF-1 α* dsRNA injection group at the early stage of the experiment ($P<0.05$) (Fig. 4D).

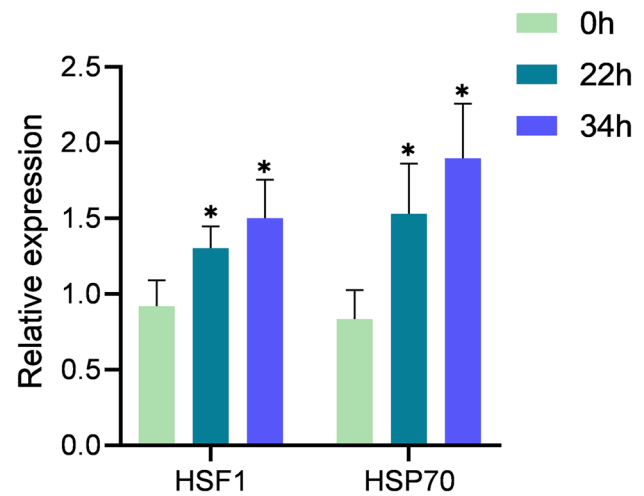


Fig. 3 Relative expression of key genes in HSR pathway of *C. gigas* under heat stress. Values were shown as the mean \pm SD ($n=3$). Asterisk indicates significant difference compared with control (0 h) (* $P<0.05$)

The HSF1 Was Directly Regulated by HIF-1 α in *C. gigas*

To verify the regulatory role of HIF-1 α on HSF1, a 2124 bp upstream sequence of the transcription start site of HSF1 was analyzed using dual-luciferase reporter assays. The results suggested that both of *HIF-1 α* and *HIF-1 α -like5* significantly activated the transcriptional activity of HSF1 ($P<0.05$) (Supplementary Fig. 1). To further determine the core promoter region, a series of plasmids with the truncated fragments of the HSF1 promoter containing different numbers of predicted HREs was constructed (Fig. 5A and Supplementary Fig. 2). The relative luciferase activity in the cells that was transfected with plasmids containing HREs of HSF1, and ORF of *HIF-1 α* was significantly higher than that in negative controls ($P<0.05$) (Fig. 5B). Similar result was observed in the cells containing HREs of HSF1 and ORF of *HIF-1 α -like5* (Fig. 5C). The position -380 and -180 of the HSF1 promoter with two binding sites was identified as the core region for *HIF-1 α* and *HIF-1 α -like5* transcription regulation since the highest relative luciferase activity was observed ($P<0.05$) (Fig. 5B and C).

Knocking Down of HIF-1 α Decreased the Survival of *C. gigas* Under Heat Stress

In order to investigate whether the expression of HIF-1 α genes was associated with thermal tolerance of *C. gigas* under heat stress, we examined the survival of oysters under heat stress with in vivo RNAi to knock down the transcriptional levels of HIF-1 α genes. The survival rate of *C. gigas* under heat stress at 24 h in PBS group, *HIF-1 α* dsRNA group, and *HIF-1 α -like5* dsRNA group was 47%,

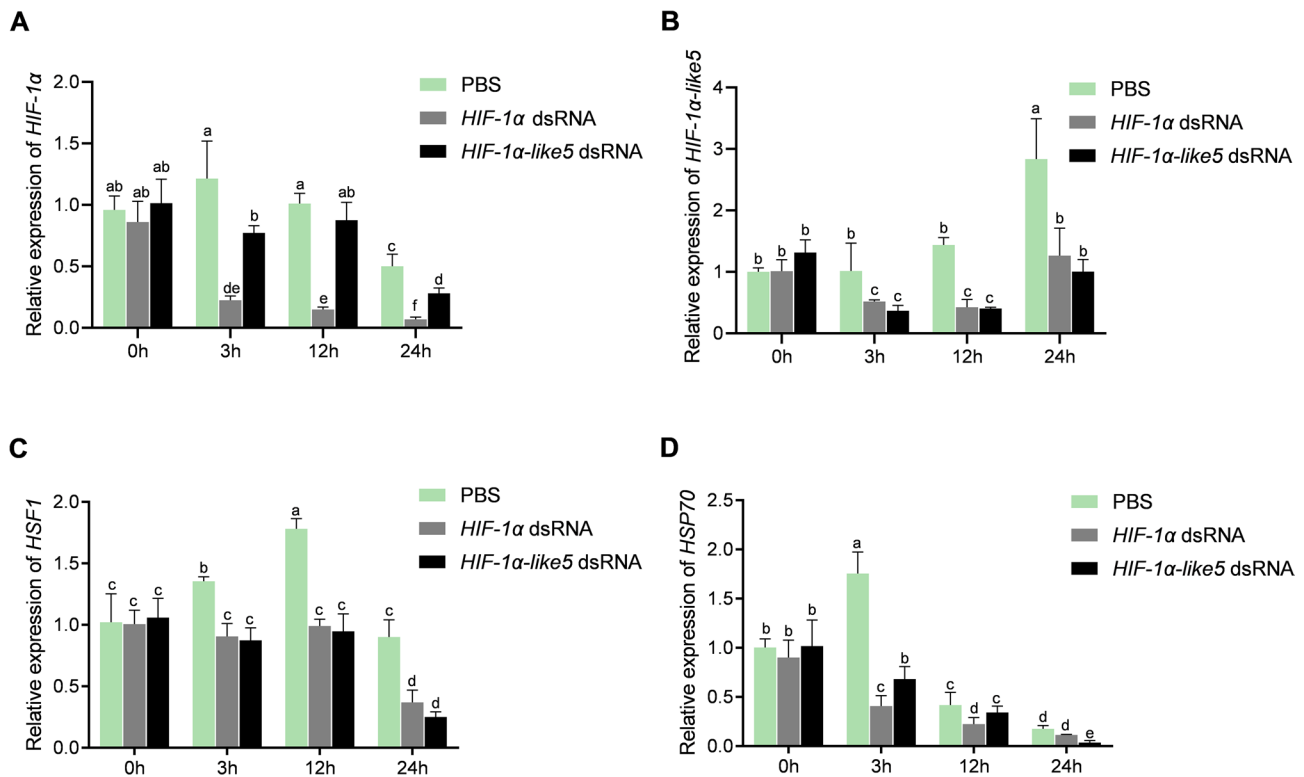


Fig. 4 Knocking down of HIF-1 α - and HIF-1 α -like5 altered expression of HIF-1 α - and HSR-related genes. Relative expression of **A** HIF-1 α , **B** HIF-1 α -like5, **C** HSF1, and **D** HSP70 in *C. gigas* under

heat stress after knocking down of HIF-1 α genes. Different letters on the error bars represent significant differences (mean \pm SD, $n=3$, $P<0.05$)

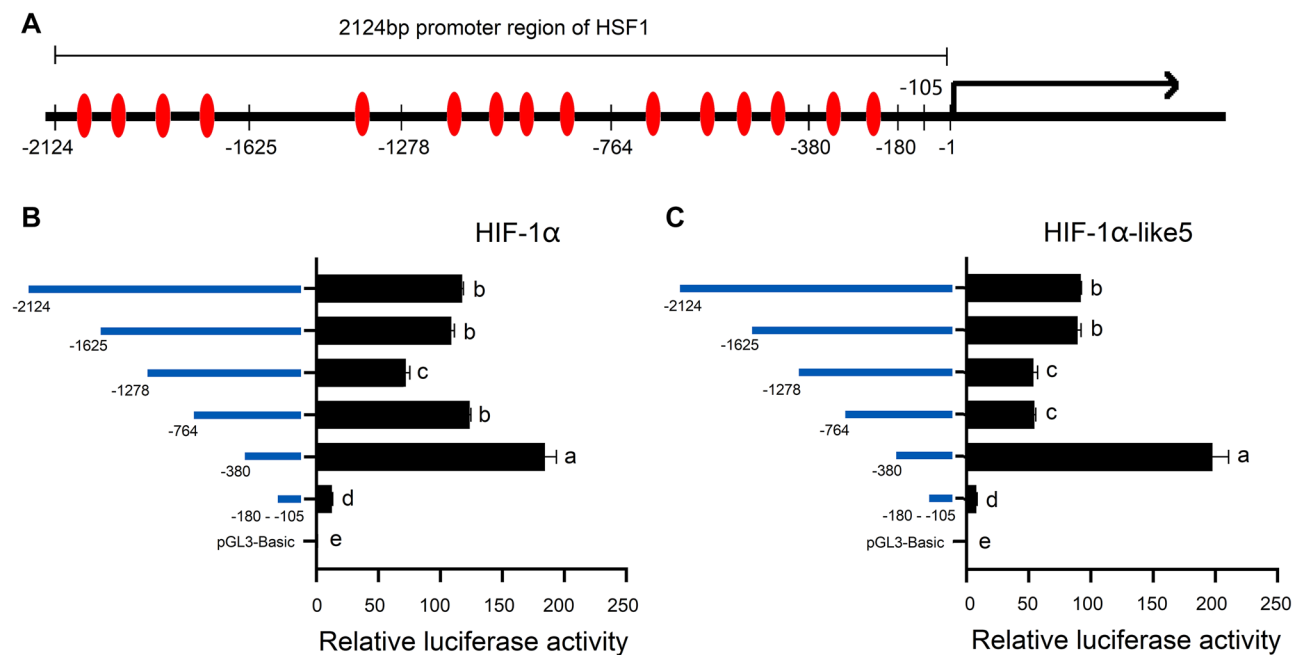


Fig. 5 Regulation of HIF-1 α on HSF1 promoter in *C. gigas*. **A** Schematic presentation of the putative binding sites of HIF-1 α in HSF1 promoter region (2124 bp) in *C. gigas*. **B** Relative luciferase activity in HEK293T cells containing different deletion constructs of HSF1

promoter and HIF-1 α . **C** Relative luciferase activity in HEK293T cells containing different deletion constructs of HSF1 promoter and HIF-1 α -like5. Data were presented as means \pm SD ($n=3$). Different letters on the bar indicate significant difference ($P<0.05$)

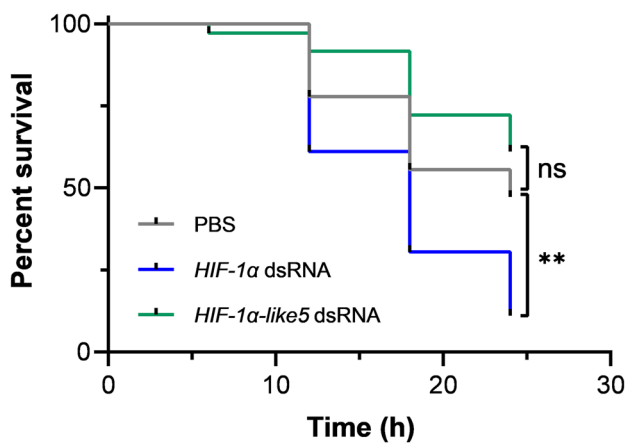


Fig. 6 Knocking down of HIF-1 α affected the survival rate of *C. gigas* under heat stress. Asterisk indicates significant difference ($n=36$, ** $P<0.01$); ns, not significant

11% and 66%, respectively (Fig. 6). The *HIF-1 α* dsRNA injection significantly reduced the survival rate of *C. gigas* under heat stress compared with the PBS and *HIF-1 α -like5* dsRNA group ($P<0.01$). Although there was no significant difference between PBS and *HIF-1 α -like5* dsRNA group, the mortality was firstly observed in the *HIF-1 α -like5* dsRNA group after heat stress.

Discussion

Environmental temperature affects the physiological and biochemical processes of organisms (Tomanek 2014). Ocean temperature rising due to global warming has posed a serious threat to the marine animals, especially those widely distributed in tidal zones, causing ecological problems and economical losses (Barbosa Solomieu et al. 2015; Gunderson and Stillman 2015; Khan et al. 2020). The HSR is an evolutionarily conserved mechanism to protect cells from heat stress which contributes to thermal tolerance of marine organisms (Clark et al. 2008; Deka et al. 2016; Dong et al. 2011; Jeremias et al. 2018; Tomanek 2010). HSR and hypoxia response are interactive cellular processes during heat stress (Ely et al. 2014; Hu et al. 2022; Huo et al. 2019; Khan et al. 2020). The transcription factor, HIF-1 α , coordinately activates multiple functional genes to promote cell survival under direct or indirect hypoxia (Bailey and Nathan 2018; Krejčová et al. 2019; Sun et al. 2016; Wang et al. 2016). Considering that, we investigated the involvement of HIF-1 α in response to heat shock in *C. gigas*, which is a widely studied model of marine bivalves (Song et al. 2019; Zhang et al. 2012).

Previous studies have reported the involvement of HIF-1 α in heat adaptation in invertebrates (Cai et al. 2014; Klumpen et al. 2017; Treinin et al. 2003). In this study, we identified

a total of six HIF-1 α genes in *C. gigas* genome, all of which contain classic conserved domains of HIF-1 α family and have a closer homology to the clade of HIF-1 α in vertebrates (Dengler et al. 2014; Wang et al. 1995). Since the gill is the respiratory and immune organ of bivalves and high temperature would cause damages on the gill tissues and affect its function (Tomanek 2012; Li et al. 2017), we further conducted the expression analysis of HIF-1 α in gills of *C. gigas* under chronic heat stress. The results showed that *HIF-1 α* and *HIF-1 α -like5* were greatly induced, which were consistent with previous observation in marine mollusks (Cai et al. 2014; Kawabe and Yokoyama 2012). These results suggested that the two members of HIF-1 α could be functionally important for *C. gigas* in response to high temperature. Knocking down of *HIF-1 α* or *HIF-1 α -like5* significantly decreased the expression levels of HSF1 and HSP70, which were the marker genes of HSR, indicating that the expression of HIF-1 α was associated with activation of HSR in *C. gigas* under heat stress. This result was consistent with previous studies in which the HIF-1 α was considered as crucial regulator for the activation of HSR (Agarwal and Ganesh 2020; Ali et al. 2011). The HIF-1 α /HSF1 signaling pathway in large yellow croaker and oyster under hypoxia has been reported (Kawabe and Yokoyama 2011; Luo et al. 2021). Therefore, a potential regulation of HIF-1 on HSF1 in *C. gigas* in response to heat stress was speculated. As a transcription factor, HIF-1 α activates gene transcription by binding to HREs in the promoter region of target genes (Dengler et al. 2014; Kaluz et al. 2008). In previous studies, several putative HREs (a core 5'-RCGTG sequence) were identified in a region of -1328 bp before the transcription start site of HSF1 in *C. gigas* (Kawabe and Yokoyama 2011). In this present study, the luciferase assay experiment revealed that two HREs located at position -360 to -180 of HSF1 promoter were both critical for the transcriptional regulation of *HIF-1 α* and *HIF-1 α -like5* in *C. gigas*. It has been reported that functional HREs tended to localize at the proximal promoters of target genes and the proximal functional elements were necessary and sufficient to regulate the transcriptional activity (Ali et al. 2011; Dengler et al. 2014). These results supported the direct regulatory relationship between HIF-1 α and HSF1 in *C. gigas* under heat stress.

The survival of *C. gigas* under heat stress was decreased after knocking down of HIF-1 α gene, which provided further support on the regulation of HSP via HIF-1 α -mediated signaling pathway. All of these results may imply the potential roles of the HIF-1 α genes in the thermal tolerance of *C. gigas*. Heat stress could cause tissue damages and result in a series of physiological responses in oyster through induction of oxidative stress and energy trade-off (Li et al. 2021; Nash et al. 2019). Multiple cellular response pathways were involved in the protection processes from heat stress, in which the HSR and hypoxia response were the most studied

interactive processes in organisms under heat stress (Ely et al. 2014; Hu et al. 2022; Huo et al. 2019). Moreover, the HIF-1 α occupied an important position in the signaling pathways that regulated various physiological functions (Liang et al. 2022; Luo et al. 2021). Therefore, we reasoned that the *HIF-1 α* served as one of the critical regulators for *C. gigas* in response to heat stress.

Conclusion

In this study, a total of six HIF-1 α genes were identified in *C. gigas* genome and were investigated for their potential contribution in response to heat stress. The *HIF-1 α* and *HIF-1 α -like5* were highly induced in *C. gigas* under chronic heat stress. The RNAi experiment in vivo and dual-luciferase reporter assay demonstrated the direct regulation of HIF-1 α on the transcriptional activity of HSF1, suggesting the critical role of HIF-1 α in contributing to thermal tolerance of *C. gigas* under heat stress. The present study for the first time revealed the involvement of HIF-1 α /HSF1/HSP70 pathway in response to heat stress in the oyster and provided an insight into adaptive mechanism of bivalves in face of ocean warming.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10126-023-10231-6>.

Author Contribution S.L. conceived the study and obtained the funding. H.F., Y.L., and J.T. performed the experiment. H.F., B.Y., and Y.L. analyzed the data. F.H. drafted the manuscript, and S.L. revised the manuscript. Q.L. supervised the work. All authors reviewed manuscript.

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Declarations

Ethics Approval The *C. gigas*, *C. angulata*, and their reciprocal hybrids are neither an endangered nor protected species. All experiments in this study were conducted according to national and institutional guidelines.

Competing Interests The authors declare no competing interests.

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