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Silencing of HIF-1 in WSSV-infected white shrimp: Effect on viral load and antioxidant enzymes

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ABSTRACT

Hypoxia inducible factor-1 (HIF-1) is a transcriptional factor that induces genes involved in glucose metabolism. HIF-1 is formed by a regulatory α -subunit (HIF-1 α) and a constitutive β -subunit (HIF-1 β). The white spot syndrome virus (WSSV) induces a shift in glucose metabolism and oxidative stress. HIF-1 α is associated with the induction of metabolic changes in tissues of WSSV-infected shrimp. However, the contributions of HIF-1 to viral load and antioxidant responses in WSSV-infected shrimp have been not examined. In this study, the effect of HIF-1 silencing on viral load and the expression and activity of antioxidant enzymes (superoxide dismutase-SOD, glutathione S-transferase-GST, and catalase) along with oxidative damage (lipid peroxidation and protein carbonyl) in tissues of white shrimp infected with the WSSV were studied. The viral load increased in hepatopancreas and muscle after WSSV infection, and the accumulative mortality was of 100% at 72 h post-infection. The expression and activity of SOD, catalase, and GST decreased in each tissue evaluated after WSSV infection. Protein carbonyl concentrations increased in each tissue after WSSV infection, while lipid peroxidation increased in hepatopancreas, but not in muscle. Silencing of HIF-1 α decreased the WSSV viral load in hepatopancreas and muscle of infected shrimp along with shrimp mortality. Silencing of HIF-1 α ameliorated the antioxidant response in a tissue-specific manner, which translated to a decrease in oxidative damage. These results suggest that HIF-1 is essential for restoring the antioxidant response, which counters the oxidative injury associated with WSSV infection.

1. Introduction

Hypoxia inducible factor-1 (HIF-1) is a transcriptional factor formed by a regulatory α -subunit (HIF-1 α) that regulates genes involved in glucose metabolism (Semenza, 1998, 1999). In the white shrimp *Litopenaeus vannamei*, HIF-1 α subunits is induced during hypoxia in a tissue-specific manner (Soñanez-Organis et al., 2009) and their silencing demonstrated that key glycolytic genes (hexokinase-HK, phosphofructokinase-PFK, fructose 1,6-bisphosphatase-FBP, lactate dehydrogenase-LDH) are regulated via HIF-1 pathway during hypoxia (Cota-Ruiz et al., 2016; Soñanez-Organis et al., 2010, 2011, 2012).

The White Spot Syndrome Virus (WSSV) (genus *Whispovirus*, family *Nimaviridae*) is a double-stranded DNA virus that causes 100% accumulated mortality within 10 days of infection on shrimp farms (van

Hulsten et al., 2001; Yang et al., 2001). WSSV induces metabolic shifts (Chen et al., 2011; Hernández-Palomares et al., 2018; Su et al., 2014) and oxidative stress from reactive oxygen species (ROS) production (Liu et al., 2010; Mohankumar and Ramasamy, 2006) in infected shrimp. ROS are highly reactive and unstable molecules that contribute to the chemical defense against pathogens. Overproduction of ROS or a deficiency in the antioxidant defense mechanisms may lead to oxidative stress that, in addition to causing cellular damage, may limit the virus replication during infection of the host (Schwarz, 1996; Valyi-Nagy and Dermody, 2005). Cells have antioxidants defenses mechanisms to balance ROS overproduction and oxidative stress, which includes non-enzymatic molecules (e.g. glutathione, vitamins, and uric acid) and enzymes (e.g. superoxide dismutase-SOD, catalase, glutathione S-transferase-GST and glutathione peroxidase (GPx) (Blokhina et al.,

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2003; Nordberg and Arner, 2001; Valko et al., 2007).

In shrimp hemocytes WSSV induced aerobic glycolysis for successful viral replication, restored host redox balance, and countered ROS production via the phosphatidylinositol 3 kinase/Akt/mammalian target of rapamycin (PIK3-Akt-mTOR) pathway (Chen et al., 2011; Chen et al., 2016; Su et al., 2014). Recently, we demonstrated that HIF-1 α is up-regulated during the WSSV infection contributing to the induction of LDH expression and glucose metabolism in energetically active tissues in shrimp (Hernández-Palomares et al., 2018). However, the effect of HIF-1 silencing on viral load along with the antioxidant response has not been examined. In this work, we report the tissue-specific effect of HIF-1 silencing over the viral load, along with the expression and activity of antioxidant enzymes (SOD, catalase, and GST) and oxidative damage (protein carbonyl and lipid peroxidation) in WSSV-infected shrimp.

2. Materials and methods

2.1. Silencing of HIF-1 and WSSV infection

Juvenile *L. vannamei* shrimp (15 ± 2 g) were obtained from a shrimp farm located in the state of Sonora, México, and acclimated during four weeks to controlled temperature (between 28 °C and 30 °C), salinity (35 ppt), recirculating-water, aeration, and were fed to 3% of their biomass with camaronina 35 (Purina). Seventy healthy shrimp and free of specific pathogens were used for this study. The inoculum of WSSV was obtained by homogenizing the muscle of WSSV-infected moribund shrimp in saline buffer (20 mM Tris, 400 mM NaCl; pH = 7.4). The homogenate was centrifuged at 10,000 rpm (9 min 4 °C) and the supernatant was filtered using sterile filters (0.8 μ m pore). The experimental inoculum was adjusted to 141, 416 copy number of WSSV/100 μ L such that the cumulative mortality reached approximately 100% between 2 and 3 days after WSSV exposure.

The double-stranded RNA (dsRNA) for HIF-1 α was synthesized from the regions corresponding to the positions 200–1036 of their homology for *L. vannamei* nucleotide sequence (GenBank accession number [FJ807918](#)) using the RiboMAX® Large Scale RNA Production Systems kits (Promega, Madison, WI, USA). The formation of the dsRNA was quantified by UV spectrophotometry and analyzed by agarose gel electrophoresis. The study was performed by injecting shrimp intramuscularly as follows: (1) saline control group (SS, $n = 10$), (2) WSSV group (100 μ L WSSV inoculum, $n = 30$ total, $n = 10$ /time point), and (3) WSSV + dsRNA group (100 μ L inoculum + 15 μ g of dsRNA HIF-1 α simultaneously, $n = 30$ total, $n = 10$ /time point). The dsRNA injection was effective for silencing HIF-1 α as previously demonstrated for this experimental group (Hernández-Palomares et al., 2018).

2.2. Sample collection and preparation

Hepatopancreas and muscle ($n = 7$) were collected from the 3 groups as follows: A) SS group at 24 h after starting the experiment, B) WSSV group at 24 and 48 h post-infection, and C) WSSV/dsRNA group at 24, 48, and 72 h post-treatment. Our initial intent was to collect tissues from the WSSV group at 72 h, but this group experienced 100% mortality by that time point. An aliquot of each tissue was separated for the individual isolation of genomic DNA (gDNA, using the PureLink® Genomic DNA Mini Kit, Invitrogen, Carlsbad, CA, USA), total RNA (tRNA, using the TRIzol reagent, Invitrogen, Carlsbad, CA, USA), and cytoplasmic protein (using the NE-PER protein extraction kit, Pierce, Rockford, IL, USA). The remaining tissues were immediately frozen in liquid nitrogen and kept at -80 °C for later analyses.

The gDNA and tRNA concentrations and integrities were analyzed by measuring the absorbance at 260/280 nm and by 1% agarose gel electrophoresis. Contamination of genomic DNA in tRNA was eliminated by digestion with RQ1 RNase-Free DNase (Promega). Total protein concentration was measured using the Bradford protein assay (Bio-

Rad Laboratories, Hercules, CA, USA). All samples were run in triplicate.

2.3. Absolute quantification of the number of copies of WSSV genomic DNA

The quantification of WSSV was done by quantitative PCR (qPCR) using specific primers for the WSSV VP-28 major envelope gene. qPCR reactions for each tissue sample were run by duplicate in the Step One Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) in a final volume of 15 μ L containing 7.5 μ L of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), 5.1 μ L of H₂O, 0.2 μ L of each primer (20 μ M), and 50 ng of gDNA. After an initial denaturing step at 94 °C for 10 min, amplifications were performed for 40 cycles at 94 °C for 15 s and 63 °C for 1 min with a single fluorescence measurement and a final melting curve program increasing 0.3 °C each 20 s from 60 to 95 °C. Standard curves from serial dilutions (1.8×10^{-3} to 1.8×10^{-7} ng μ L⁻¹) of VP-28 PCR fragments were generated and used to calculate the number of WSSV copies using the formulations as described previously (Mendoza-Cano and Sanchez-Paz, 2013).

2.4. Quantification of antioxidant genes

Total DNA-free RNA (2.5 μ g) was used to synthesize one cDNA for each sample using oligo-dT and the GoScript™ Reverse Transcriptase kit (Promega). Specific primers for each gene were designed based on the nucleotide sequences deposited in GenBank database (Table 1). The qPCR reactions were performed separately for each gene and normalized by the expression of the ribosomal protein L8. Two PCR reactions for each cDNA (2 data/sample and 14 data/group) were run (Step One Real-Time PCR Systems, Applied Biosystems) in a final volume of 15 μ L containing 7.5 μ L of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), 5 μ L of H₂O, 0.25 μ L of each primer (20 μ M), and 1 μ L of cDNA (equivalent to 125 ng of the original tRNA). After an initial denaturing step at 94 °C for 10 min, amplifications were performed for 40 cycles at 94 °C for 15 s and 63 °C for 1 min with a single fluorescence measurement, and a final melting curve program increasing of 0.3 °C each 20 s from 60 to 95 °C. Positive and negative controls were included for each gene. Standard curves for each gene and ribosomal protein L8 were run to determine the efficiency of amplification using dilutions from 5×10^{-3} to 5×10^{-8} ng μ L⁻¹ of PCR fragments.

2.5. Biochemical analysis

2.5.1. Superoxide dismutase (E.C. 1.15.1.1)

Total SOD activity was measured at 25 °C by mixing 590 μ L of the work solution [50 mM sodium carbonate, 0.1 mM xanthine, 0.025 mM of nitro blue tetrazolium (NBT), 0.1 mM of EDTA], 1 μ L of 0.1 mM xanthine oxidase and 10 μ L of cytoplasmic protein extract or blank (potassium phosphate buffer). All measurements were performed in triplicate measuring the absorbance of each sample at 560 nm in a Jenway 6705 UV–vis spectrophotometer for a total of 5 min at 25 °C.

Table 1

Primers used for the qPCR of each gene.

Primer name	Nucleotide sequences (5'-3')	GeneBank accession number	Product size (base pairs)
<i>CatFw</i>	GCGTGACAAGTGTGCAGAGC	AY518322.1	190
<i>CatRv</i>	GCTCCCTTAGCATGCACAACC		
<i>GSTFw</i>	CACGGGCACTGAGTTCGAGG	AY573381.2	208
<i>GSTRv</i>	CGTGATGTCTGTCAAGTTG		
<i>MnSODFw</i>	GGAAGCTTACATCTCCATCC	DQ005531.1	227
<i>MnSODRv</i>	GCATGAGCACCTCGTTCCTC		
<i>L8F</i>	GTCTACTGCGGCAAGAAGGC	DQ316258.1	197
<i>L8R</i>	CCTGAAGGGAGCTTTACACG		

SOD activity is reported as the amount of enzyme needed to inhibit the reaction of O_2^- with NBT by 50%, and was expressed in $U \mu g \text{ protein}^{-1}$.

2.5.2. Glutathione-S-transferase (E.C. 2.5.1.18)

GST activity was measured at 25 °C by mixing 459 μL of work solution [100 mM phosphate buffer pH 7.0, 60 mM EDTA pH 10, 10 mM 1-chloro 2,4-dinitrobenzene (CDNB)], 25 μL of 10 mM glutathione and 25 μL of cytoplasmic protein extract or blank (work solution). Activity was calculated following the formation of thioether–glutathione–dinitrobenzene from the reaction between glutathione and CDNB. GST activity was expressed in $U \mu g \text{ protein}^{-1}$ and is defined as the amount of enzyme that synthesizes 1 μmol of product min^{-1} .

2.5.3. Catalase (E.C. 1.11.1.6)

Catalase activity was measured using the Catalase Assay Kit (Cayman Chemical, 707002) following the manufacture instructions. All measurements were performed in triplicate following the absorbance of each sample at 540 nm in a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Catalase activity is reported as the amount of enzyme required for the production of 1.0 nmol of formaldehyde per minute at 25 °C, and was expressed in $U \mu g \text{ protein}^{-1}$.

2.5.4. Protein carbonyl and lipid peroxidation

Oxidative damage to proteins and lipids were determined using the Protein Carbonyl Content Assay Kit (MAK094, SIGMA-ALDRICH) and Lipid Peroxidation (MDA) Assay kit (ab118970, abcam), respectively, following the manufacture instructions. All measurements were performed in triplicate measuring the absorbance of each sample at 375 nm and 540 nm for protein carbonyl and lipid peroxidation, respectively, in a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific). The results are expressed as nmol of carbonyl $\mu g \text{ protein}^{-1}$ and μM of malondialdehyde (MDA) for protein carbonyl and lipid peroxidation, respectively.

2.6. Statistics

Initially, data were tested for normality and homogeneity of variance. Differences for each group were detected by one-way ANOVA with Bonferroni post-hoc test using the saline solution-injected shrimp as control. Means ($\pm$ s.e.m.) were considered statistically different at $P < 0.05$ (STATISICAL 8 software, StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Silencing of HIF-1 α decreased the mortality and WSSV genomic DNA copy number in WSSV infected shrimp

The accumulative mortality of the WSSV group was 65% and 100% at 48 and 72 h post-infection, respectively, while mortality of the WSSV/dsRNA group was 15% and 20% at 48 and 72 h post-treatment, (Fig. 1). The largest number of WSSV copies detected was 2.8×10^5 and 1.5×10^8 in muscle and 1.8×10^5 and 7.4×10^5 in hepatopancreas of WSSV group at 24 h and 48 h post-infection, respectively. In contrast, the number of WSSV copies decreased 99% and 98% in muscle of WSSV/dsRNA group at 48 h and 72 h post-treatment, respectively, compared to WSSV group at 48 h. No differences were detected between the WSSV and WSSV/dsRNA groups at 24 h post-treatment. In hepatopancreas, the WSSV copies decreased 60% in the WSSV/dsRNA at 24 h post-treatment, compared to WSSV group at 24 h, while in the WSSV/dsRNA group copies decreased 95% and 86% at 48 h and 72 h, respectively, compared to WSSV group at 48 h (Fig. 2).

Fig. 1. Gene silencing of shrimp HIF-1 α decreases mortality after WSSV infection. Cumulative mortalities of shrimp were observed after WSSV infection, and ameliorated after injection with dsRNA corresponding to HIF-1 α . Mortality was measured in each treatment group ($n = 30$) and was recorded every 24 h post-challenge.

3.2. Antioxidants genes are regulated in a tissue-specific manner during WSSV infection

The expression of SOD decreased 2- and 7-fold in hepatopancreas of WSSV group at 24 and 48 h post-infection, respectively, compared to SS group, while in the WSSV/dsRNA group the expression increased 4-fold at 24 h and decreased 1.5-fold at 48 h and 72 h. In muscle, SOD expression increased 2.3-fold in the WSSV group at 24 h post-infection compared to SS group and decreased 1.5-fold in the WSSV/dsRNA group at 24 h, 48 h, and 72 h post-treatment (Fig. 3A). The expression of GST decreased 4.3- and 30-fold in hepatopancreas of the WSSV group at 24 h and 48 h post-infection compared to the SS group, respectively. In the WSSV/dsRNA group, GST expression increased 5.5- and 3-fold in the WSSV/dsRNA group at 24 h and 48 h post-treatment compared to the SS group, with the level at 72 h similar to the SS group. In contrast, the expression of GST decreased 1.3- and 2.4-fold in muscle of the WSSV at 24 h and 48 h post-infection compared to the SS group, respectively. The expression of GST also decreased 6-, 1.5- and 1.8-fold in the WSSV/dsRNA group at 24 h, 48 h and 72 h after treatment (Fig. 3B). The expression of catalase decreased 7- and 30-fold in hepatopancreas of the WSSV group at 24 h and 48 h, respectively, post-infection compared to the SS group, while in the WSSV/dsRNA group, levels increased 4- and 2-fold at 24 h and 48 h, respectively, decreasing to basal levels at 72 h. In muscle, the expression of catalase increased 2.4-fold in the WSSV group at 48 h post-treatment compared to the SS group, while in the WSSV/dsRNA group catalase expression decreased at 24 h and increased 1.6- and 2.2-fold at 48 h and 72 h, respectively, after treatment (Fig. 3C).

3.3. Antioxidant enzyme activities are regulated in a tissue-specific manner during WSSV infection

SOD activity increased 2-fold in hepatopancreas and muscle of the WSSV group at 24 h or 48 h post-infection, respectively, compared to the SS group. In the WSSV/dsRNA group, SOD activity increased 2.3-fold in hepatopancreas at 24 h post-treatment compared to the SS group and returned to basal level at 48 h and 72 h post-treatment. In muscle, SOD activity returned to basal levels at 24 h and 48 h post-treatment and increased 2.3-fold at 72 h post-treatment (Fig. 4A). GST activity

Fig. 2. Tissue-specific WSSV loads in hepatopancreas and muscle from WSSV-infected shrimp and HIF-1 α silencing. Real-time qPCR was used to measure the number of copies of WSSV genomic DNA in WSSV-challenged shrimp that had been preinjected with double-stranded RNAs corresponding to HIF-1 α . Asterisks denote significant ($P < 0.05$) differences from WSSV group.

increased 1.2-fold and decreased 2-fold in hepatopancreas of WSSV group at 24 h and 48 h post-infection, respectively, compared to the SS group, while in the WSSV/dsRNA group GST activity decreased 1.2-fold at 24 h, 48 h, and 72 h post-treatment. In muscle, GST activity decreased 1.8-fold in the WSSV group at 24 h and 48 h post-infection compared to the SS group, while in the WSSV/dsRNA group GST activity remained at basal levels at 24 h and 48 h before increasing 2.3-fold at 72 h (Fig. 4B). The activity of CAT did not change in hepatopancreas of WSSV group, but increased 2-fold in the WSSV/dsRNA group compared to SS group. In muscle, CAT activity increased 1.5-fold in the WSSV and WSSV/dsRNA groups (Fig. 4C).

3.4. Silencing of HIF-1 α decreased the oxidative damage in WSSV infected shrimp

Protein carbonyl increased 2-fold in hepatopancreas of WSSV group at 48 h post-infection compared to the SS group, and remained at basal levels in the WSSV/dsRNA group at 24 h, 48 h, and 72 h post-treatment. In muscle, protein carbonyls increased 2-fold in the WSSV group at 24 h and 48 h compared to the SS group, while in the WSSV/dsRNA group protein carbonyls remained at basal level at 24 h, 48 h, and 72 h (Fig. 5A). Lipid peroxidation increased 2.5- and 6-fold in hepatopancreas of WSSV group at 24 h and 48 h post-infection, respectively,

Fig. 3. Mean ($\pm$ s.e.m.) Tissue-specific expression of antioxidant genes (SOD, CAT, GST and GPx) in hepatopancreas and muscle from WSSV-infected shrimp and HIF-1 α silencing. mRNA levels were determined by quantitative RT-PCR and compared to ribosomal 18 levels, $n = 5$. Asterisks denote significant ($P < 0.05$) differences from SS group.

compared to the SS group and increased 3.4-, 3- and 3.2-fold in the WSSV/dsRNA at 24 h, 48 h, and 72 h post-treatment, respectively. In muscle, lipid peroxidation decreased 3.3- and 4.6-fold in the WSSV group at 24 h and 48 h post-infection, respectively, compared to the SS

Fig. 4. Mean ($\pm$ s.e.m.) Antioxidant enzyme activity (SOD, CAT, GST and GPx) in hepatopancreas and muscle from WSSV-infected shrimp and HIF-1 α silencing. Asterisks denote significant ($P < 0.05$) differences from SS group. All enzyme activities are expressed in Units/ μ g of protein.

group, while in the WSSV/dsRNA group levels decreased 3- and 6-fold at 48 h and 72 h, respectively (Fig. 5B).

4. Discussion

The WSSV infection impairs aerobic glycolysis for successful viral replication, restoration of host redox balance, and counters ROS

Fig. 5. Mean ($\pm$ s.e.m.) Protein carbonyl content (A) and lipid peroxidation (B) in hepatopancreas and muscle from WSSV-infected shrimp and HIF-1 α silencing. Asterisks denote significant ($P < 0.05$) differences from SS group.

overproduction via the PI3K-Akt-mTOR pathway (Chen et al., 2011; Chen et al., 2016; Su et al., 2014). Recent studies have demonstrated that HIF-1 regulates aerobic glycolysis through induction of the expression and activity of LDH in highly energetic tissues in white shrimp when infected with WSSV (Hernández-Palomares et al., 2018). However, the effect on viral load and oxidative stress defense by the silencing of HIF-1 has not been defined in tissues from WSSV-infected shrimp. In this work, we demonstrated that the silencing of HIF-1 decreased the mortality of shrimp along with the WSSV viral load and that WSSV infection up-regulated the expression and activity of antioxidant enzymes in a tissue-specific manner. Also, the silencing of the HIF-1 counteracted the effects produced by the WSSV infection on the expression and activity of antioxidant enzyme, and of the oxidative

damage as indicated by carbonylation of proteins.

WSSV viral load is associated with high mortalities on the major penaeid shrimp species. The viral load varies significantly with tissue type (on the order of 10^5 – 10^{10} copies), and increased shrimp mortalities occur between 2 and 5 days post-infection. Under experimental infections conditions, the viral load and mortality depend on the route of infection (injection or per os), the inoculum concentration, and the time of sampling (Oidtmann and Stentiford, 2011). In WSSV-infected white shrimp, the highest viral load was detected in gills (1.2×10^9 copies) followed by muscle (1.9×10^8 copies) and hepatopancreas (9.0×10^7 copies) (Durand and Lightner, 2002). Our results showed that both viral load and shrimp mortalities are similar to those reported in WSSV-infected shrimp, validating the experimental design and procedures.

Several studies describe that the mRNA expression and activity of antioxidant enzymes of shrimp are differentially regulated in a tissue-specific manner during the WSSV infection. The expression of catalase increased in hemocyte and hepatopancreas of Chinese shrimp *Fenneropenaeus chinensis* at 14 h and 37 h post-infection with the WSSV (Zhang et al., 2008). Also GST and GPx increased in hemocyte and hepatopancreas of *Exopalaemon carinicauda* at 3 h and 6 h post-infection (Duan et al., 2013), and mitochondrial manganese SOD (mMnSOD) increased in hemocytes of *F. chinensis* between the 48 h post-infection (Zhang et al., 2007), GPx increased in hemocytes of *Penaeus monodon* at 48 h post-infection (Kuan-Fu et al., 2010). SOD isoforms were induced differentially in hemocytes at the first hour post-infection (Gómez-Anduro et al., 2012; Gómez-Anduro et al., 2006), decreasing at 48 h post-infection (Zhang et al., 2007). On the other hand, the activities of SOD, catalase, GST, and GPx decreased in haemolymph and tissues of *P. monodon* (Mathew et al., 2007) and *L. vannamei* (Parrilla-Taylor et al., 2013) at 48 h post-infection with the WSSV.

Reductions in the expressions and activities of antioxidant enzyme in WSSV-infected shrimp resulted in oxidative stress and tissue damage, systematic failure, and ultimately sudden death. WSSV infection increased lipid peroxidation in *F. indicus* (Mohankumar and Ramasamy, 2006; Subramanian and Philip, 2013) and *P. monodon* (Mathew et al., 2007; Rameshthangam and Ramasamy, 2006), and increased both lipid peroxidation and protein carbonyls in *L. vannamei* (Parrilla-Taylor et al., 2013). The differences in antioxidant enzyme responses and oxidative damage may be specific to tissues. The hepatopancreas is a major site of ROS production, contains high levels of polyunsaturated fatty acids, and participates in the digestion, absorption, and storage of nutrients, while muscles functions in locomotion with high glycolytic and gluconeogenic capacity (Gibson and Barker, 1979). Our results corroborate these previous studies and demonstrate reduced antioxidant enzymes expressions and activities especially in the hepatopancreas in infected shrimp, resulting in oxidative damage.

The regulation of the expression of antioxidant genes is critical for ROS homeostasis in many settings. Several studies have demonstrated tissue-specific expression of the antioxidant genes in mammals through transcriptional factors such as specificity protein 1 (Sp1), activator protein (AP), and the nuclear factor E2-related factor 2 (Nrf2) (Nguyen et al., 2009; Van Remmen et al., 1998; Luo and Rando, 2003; Xu et al., 2002). However, there are not studies on the regulation of antioxidant genes in shrimp. The WSSV infection induces aerobic glycolysis in hemocytes (Chen et al., 2011; Su et al., 2014) and tissues (Hernández-Palomares et al., 2018) of white shrimp, mimicking the Warburg effect. In hemocytes in white shrimp, WSSV induce the Warburg effect via the PIK3-Akt-mTOR pathway to counter ROS overproduction and reduce the host's oxidative stress defenses for successful viral replication (Chen et al., 2016). In human cancer, ROS enhance the signaling activity of PIK3-Akt-mTOR pathway, which in return induces HIF-1 α transcription and translation under both hypoxic and normoxic conditions (Movafagh et al., 2015). We have shown previously that expression of HIF-1 α is up-regulated in tissues from WSSV-infected shrimp. Additionally, silencing of HIF-1 blocked the glycolysis produced by the WSSV infection through the down-regulation of LDH, which decreased LDH activity, glucose consumption, and lactate accumulation in highly energetic tissues of white shrimp suggesting that HIF-1 participates in the pathogenesis of this virus through the induction of metabolic changes (Hernández-Palomares et al., 2018). The decreased viral load and shrimp mortalities by the silencing of HIF-1 α demonstrated that HIF-1 contributes in the pathogenesis of WSSV through the induction of the Warburg effect that ultimately leads to the tissue-specific activation of antioxidant enzymes to balance ROS overproduction and oxidative stress.

In summary, the present study demonstrated that HIF-1 participates in the pathogenesis of WSSV infection through the regulation of their viral replication in highly energetic tissues of infected shrimp. Moreover, the blockade of viral replication by the silencing of HIF-1 α

restored antioxidant defenses that countered oxidative damage, which ultimately led to increased mortality if not ameliorated. Thus, treatments for WSSV infection in the shrimp industry should target HIF-1 to promote beneficial antioxidant balance to combat oxidative injury-induced mortality.

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