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Differential interferon system gene expression profiles in susceptible and resistant gynogenetic clones of gibel carp challenged with herpesvirus *Ca*HV

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ABSTRACT

Interferon (IFN) system plays a vital role in the first line of defense against viruses. In this study, we first identified multiple transcripts of 15 IFN system genes, including PRRs (*TLR2*, *TLR3*, *RIG-I*, and *LGP2*), PRR-mediated IFN signal pathway (*MyD88*, *MITA*, and *MAVS*), IFN regulatory factors (*IRF1*, *IRF3*, *IRF7*, and *IRF9*), IFNs (*IFNφ1* and *IFNφ3*), and ISGs (*Mx* and *viperin*), and one transcript of *TLR9* in *de novo* transcriptome assembly data of gibel carp head-kidney. Multiple nucleotide alignments and phylogenetic analysis of common region showed that the transcripts of every of the 15 IFN system genes were classified into two homologs with distinctly divergent sequences, indicating that hexaploid gibel carp may be an allo-polyploid. During *Carassius auratus* herpesvirus (*Ca*HV) infection, gibel carp resistant clone H significantly suppressed *Ca*HV replication with markedly less viral loads than those in highly susceptible clone A^+ and moderately resistant clone F. Then, qPCR analyses were performed to reveal their differential and dynamic expression changes during *Ca*HV infection in head kidney, spleen and liver among three gibel carp gynogenetic clones. Through qPCR and hierarchical clustering analysis, 8 genes, such as *RIG-Is*, *LGP2s*, *IRF1-B*, *IRF3s*, *IRF7s*, *IRF9-B*, *Mxs*, and *viperins*, were identified as candidate resistant-related genes. They remarkably increased their expression in immune tissues of three clones after *Ca*HV infection. Significantly, the up-regulation folds of these genes in clone A^+, F and H were related to their resistance ability to *Ca*HV, progressively increasing from susceptible clone to resistant clone at 1 dpi. The positive correlation to the resistance ability suggested that resistant clone H immediately triggered stronger IFN response. *IFNφ3* showed a different dynamic change and was sharply induced in moderately resistant clone F at 3 dpi. The other 5 IFN system genes (*TLR2*, *TLR3*, *TLR9*, *MyD88*, and *MITA*) maintained a low expression level after *Ca*HV challenge. Interestingly, the A or B copies/homologs of almost these IFN system genes exhibited differential transcript abundance in immune tissue after *Ca*HV challenge, suggesting A or B homologs might occur dominant or biased expression of homeologs during gibel carp evolution. These data provide candidate resistant-related genes for disease-resistance breeding of gibel carp.

1. Introduction

Gibel carp (*Carassius gibelio*) has been popularly cultured in almost the whole country of China since all-female gibel carps produced by heterologous sperm gynogenesis (termed allogynogenesis) were applied in the early 1980s (Gui and Zhou, 2010; Jiang et al., 1983). Owing to the application of several improved varieties with excellent growth performance, such as allogynogenetic gibel carp "CAS III" (clone A^+), the annual production capacity of crucian carp has rapidly increased to 2,912,258 tons in 2015 (Zhou and Gui, 2017). However, an epizootic disease caused by *Carassius auratus* herpesvirus (*Ca*HV) (Zeng et al., 2016) has threatened the culture industry and brought about enormous economic loss in main culture areas of Jiangsu province since 2009.

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Gibel carp is considered as evolutionally hexaploid with over 150 chromosomes (Li et al., 2014b, 2016; Zhou and Gui, 2002; Zhu et al., 2006). The draft genome sequence of *Cyprinus carpio* indicated that allotetraploidization (species hybridization) resulted in the tetraploidy observed in *Cyprinus carpio* (Xu et al., 2014b). The evolutionary relationship analyses of two divergent *Dmrt1* genes among *Carassius* species complex and *Cyprinus carpio* had revealed that an early polyploidy event might result in a common tetraploid ancestor of *Carassius gibelio*, *Carassius auratus auratus* and *Cyprinus carpio* before 18.49 million years ago (Mya), and a late autoploidization (genome doubling) might occur from the evolutionary branch of *Carassius auratus* at around 0.51 Mya, which lead to the current hexaploid gibel carp (Li et al., 2014b). In addition, various gynogenetic clones in cultured populations or nature regions of the Eurasian continent were discriminated by biochemical and molecular markers (Apalikova et al., 2008; Bai et al., 2011; Gao et al., 2017; Gui and Zhou, 2010; Jakovlic and Gui, 2011; Jiang et al., 2013; Sakai et al., 2009; Tsipas et al., 2009; Vetesnik et al., 2007; Wouters et al., 2012; Yang et al., 2001; Zhou et al., 2000, 2017, 2001). Significantly, these diverse gynogenetic clones exhibit remarkable differences in growth and disease resistance, which provide materials for gibel carp breeding. Through herpesvirus *Ca*HV challenge experiments, three gynogenetic clones of gibel carp, including a highly susceptible clone (A^+), a moderately resistant clone (F) and a strongly resistant clone (H), were identified. Moreover, distinct immune responses of three clones to *Ca*HV infection and a lot of differentially expressed unigenes (an assembled cDNA/transcript with unique sequence) (DEU), especially interferon (IFN) system genes, were identified from comparative transcriptomes between diseased individuals and control individuals of clone A^+, F and H, respectively (Gao et al., 2017).

IFNs play a vital role in the first line of defense against viruses. In mammals, three classes of IFNs were identified and type I IFN signaling has been well characterized (Ng et al., 2016; Sadler and Williams, 2008; Schreiber, 2017; Teijaro, 2016). Tremendous advances in identification and antiviral mechanisms of IFN system genes have been achieved in teleost (Chen et al., 2017; Langevin et al., 2013; Poynter et al., 2015; Poynter and DeWitte-Orr, 2016; Secombes and Zou, 2017; Verrier et al., 2011; Zhang and Gui, 2012; Zhu et al., 2013; Zou and Secombes, 2011, 2016). Similar to mammals, fish initiates type I IFN response through recognizing viral products by pattern recognition receptors (PRRs), then triggers IRF3/7-dependent IFN response, and finally induces type I IFN and subsequent downstream antiviral genes including Mx and viperin (Chen et al., 2017; Langevin et al., 2013; Poynter et al., 2015; Poynter and DeWitte-Orr, 2016; Secombes and Zou, 2017; Verrier et al., 2011; Zhang and Gui, 2012; Zhu et al., 2013; Zou and Secombes, 2011, 2016). About 20 IFN system genes, such as *IFN* (Yu et al., 2010), *IRF1* (Shi et al., 2008), *IRF3* (Sun et al., 2010), *IRF7* (Zhang et al., 2003), *IRF9* (Shi et al., 2012), *signal transducer and activator of transcription 1* (*STAT1*) (Zhang and Gui, 2004), *mediator of IRF3 activation* (*MITA*) (Sun et al., 2011) and *viperin* (Wang et al., 2014), were identified from UV-inactivated GCHV-infected *Carassius auratus* blastula (CAB), and their expression regulation and antiviral function have been well investigated *in vitro* (Zhang and Gui, 2012). *Carassius auratus* appears to trigger type I IFN antiviral response by similar mechanism. Interestingly, *Carassius auratus* IFN exhibits combined characters of mammalian type I IFNs and type III IFNs and can induce the expression of itself and ISGs by Jak-Stat signaling pathway (Yu et al., 2010; Zhang and Gui, 2012). However, little is known about the dynamic expression changes of IFN system genes *in vivo* in gibel carp after virus infection, particularly the difference between susceptible clone and resistant clone after *Ca*HV challenge. In the *de novo* transcriptome assembly data of gibel carp head-kidney (Gao et al., 2017), multiple DEU were annotated as the same IFN system gene. The divergent expressions of different alleles or copies of IFN system genes are largely unknown in gibel carp. Here, we first identified multiple transcripts of 15 IFN system genes and classified every IFN system gene into divergent A or B group. Then, we analyzed their differential expression profiles in head kidney, spleen and liver among clone A^+, F and H.

2. Materials and methods

2.1. CaHV infection and sample collection

6-month old individuals of gibel carp clone A^+, F and H, with 85.89 ± 2.13 g, 67.68 ± 2.16 g, 46.96 ± 2.47 g average weight respectively, were selected to challenge with herpesvirus *Ca*HV by intraperitoneal injection with 500 μL *Ca*HV viral suspension (2.915×10^8 copy number of *Ca*HV-DNA) per fish. 30 individuals of each clone were injected with equal phosphate-buffered saline solution (PBS) as the control group. Before *Ca*HV infection, apparently healthy individuals were gradually acclimatized in 150-L tanks with aerated water at 24(±1) °C and fed with commercial feed twice a day for two weeks. The amplification, identification and titration of *Ca*HV and the challenge experiment were performed as previously described (Gao et al., 2017). All fish were obtained from the GuanQiao Experimental Station, Institute of Hydrobiology, Chinese academy of sciences and animal experiments described in this paper were conducted according to the Animal Care and Use Committee of Institute of Hydrobiology, Chinese Academy of Sciences. Tissues, including head-kidney, kidney, spleen and liver, were collected from individuals before infection (0 day), infected fish at 1, 3, and 5 days post injection (dpi), respectively. All samples were preserved in RNAlater (Qiagen) and stored at −20 °C for nucleic acid extraction.

2.2. Viral DNA extraction and quantification

Three infected individuals of each clone at 1, 3, and 5dpi, and three individuals of each clone before infection were selected to evaluate viral load. Total DNA was extracted using DNA extraction kit (Promega, USA) and viral quantification was calculated by real-time PCR analysis as previously described (Goodwin et al., 2006). Briefly, a 637-bp helicase gene fragment of *Ca*HV was amplified by primers (5′-CTTTAGCGTCAGGTCCATAGAGG-3′ and 5′-CGTCAGTCCCTGGCAGAAATAAG-3′) and inserted into the pMD18-T plasmid to produce pMD-*Ca*HV (3329 bp). The concentration of pMD-*Ca*HV was determined by spectrophotometry and converted to viral loads of *Ca*HV by using an empiric formula (viral load = concentration of plasmid (g/μL) × 6.02×10^{23}/(660 u/bp × nucleotide number of plasmid)). Then, a 10-fold dilution series of pMD-*Ca*HV was used as the standard template to perform quantitative real-time PCR and to establish standard curve. The copy number of *Ca*HV-DNA of each sample were calculated through standard curve. The primers (5′-TTAGCG TCAGGTCCA AG-3′ and 5′-GGCGTGTAGAAATCA AACT-3′) and procedure of real-time PCR were designed as described (Goodwin et al., 2006; Xu et al., 2014a). All were run in triplicate.

2.3. Sequence and phylogenetic analyses

IFNφ1 and *RIG-I* cDNA sequences of zebrafish (*Danio rerio*) and common carp (*Cyprinus carpio*) were obtained from GenBank database through blast program, and the fragments with the same position of corresponding unigenes were selected for further analyses.

Multiple sequences were aligned by ClustalX program, and phylogenetic construction was adjusted by bootstrap analysis