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Short communication

Micropterus salmoides rhabdovirus (MSRV) infection induced apoptosis and activated interferon signaling pathway in largemouth bass skin cells

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ABSTRACT

Largemouth bass (*Micropterus salmoides*) rhabdovirus (MSRV) was isolated from infected juveniles of largemouth bass, and the infected fish exhibited corkscrew, irregular swimming, and crooked body. To our knowledge, the potential molecular mechanisms underlying the pathogenesis of MSRV infection remain largely unknown. In the current study, we found that MSRV infection in largemouth bass skin (LBS) cells induced typical apoptosis, evidenced by the presence of apoptotic bodies and caspase-3 activation. To further analyze the host factors involved in MSRV infection in LBS cells, the transcriptomic profiles during MSRV infection were uncovered using deep RNA sequencing technique, and several differentially expressed genes (DEGs) were validated by quantitative PCR. Our results showed that a total of 124483 unigenes were assembled. Among them, 34465 and 27273 had significant hits to those in the NR and SwissProt databases. After MSRV infection, a total of 2432 and 2480 genes which involved in multiples pathways including TNF signaling, NF-κB signaling, Toll-like receptor signaling and RIG-I signaling pathway were differentially expressed in MSRV infected LBS cells compared to mock-infected cells at 12 h, respectively. Furthermore, quantitative PCR showed that the expression levels of 9 differentially expressed genes (DEGs) related to apoptosis and interferon signaling pathway was consistent with that from transcriptomic profiles. Together, our results not only demonstrated that interferon signaling pathway and apoptosis pathway might exerted crucial roles during MSRV infection, but also provided a useful resource for subsequent investigation of other immune-related genes related to virus infection.

1. Introduction

Largemouth bass (*Micropterus salmoides*) is a species of freshwater fish native to the southeastern United States that have been introduced into different countries worldwide [1–3]. Recently, it has received considerable attentions due to the related studies of environmental toxicology and the intersex phenomenon in largemouth bass [4–6]. However, both wild and cultured largemouth bass has suffered from different diseases evoked by viruses, bacteria and parasites in recent years [7–13]. Among these pathogens, largemouth bass virus (LMBV) belonged to *Ranavirus* genus, *Iridoviridae* family and its infection evoked swollen spleen and kidneys, extensive ulcerations on the body surface and necrosis of naked muscle of diseased largemouth bass [8]. Fish infected with *Micropterus salmoides* rhabdovirus (MSRV) exhibited corkscrew, irregular swimming, and crooked body [12]. In addition, *largemouth bass reovirus* (LMBRV) and *Micropterus salmoides reovirus* (MsReV) have been isolated form wild and cultured largemouth bass in recent years [11,13].

In recent years, great efforts on generating fish genomic resources have been made in model species and economically important aquaculture species [14–16]. The fish genome information promoted the research on functional genomics, especially in fish immunology and gene regulatory networks [16]. Due to lack of genome information of largemouth bass, few immune genes were cloned and characterized up to date [17,18]. Although the expression profiles of genes in largemouth bass after challenge with nocardia seriolae were explored using de novo transcriptome sequencing [19], few reports focused on the host immune response against virus infection.

In this study, we demonstrated the transcriptomic profiles of MSRV infection in vitro using deep RNA sequencing technique. Moreover, the expression patterns of several genes involved in interferon immune response and apoptotic pathway was confirmed using quantitative PCR. Our results will not only provide the information of a large number of candidate genes in immunology, but also contributed greatly to understanding the potential molecular mechanism of fish rhabdovirus pathogenesis.

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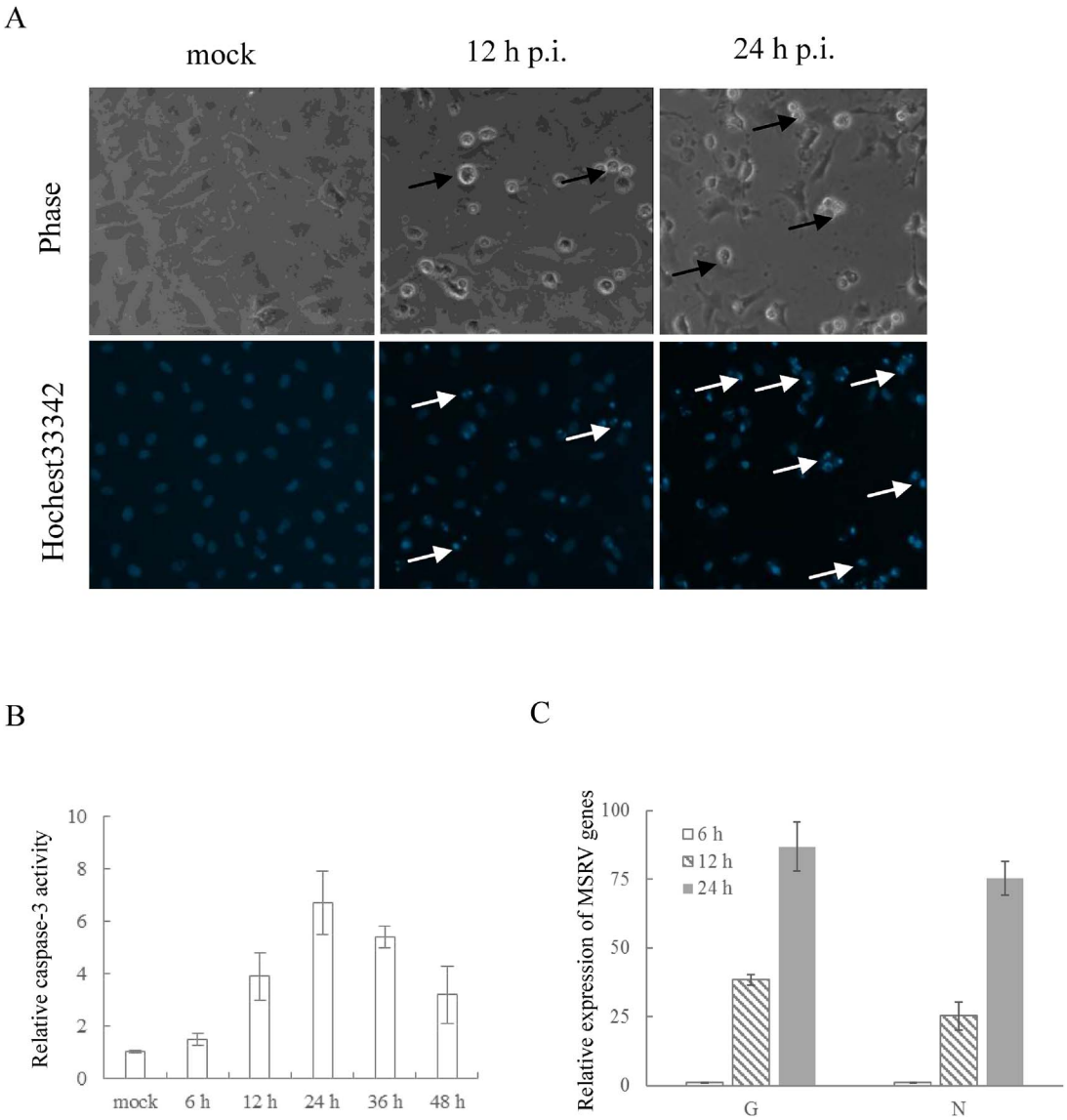

Fig. 1. MSRV replicated well and induced apoptotic cell death in LBS cells. (A) MSRV infection induced apoptotic bodies in LBS cells. Black arrows showed rounded cells in LBS cells, and the white arrows indicated the apoptotic bodies in LBS cells. (B) The caspase-3 activity induced by MSRV infection in LBS cells. (C) The expression of viral genes during MSRV infection. The relative expression level of nucleoprotein (N) and glycoprotein (G) were determined using qRT-PCR. The transcription values were normalized to that of β-actin, and the relative mRNA levels of the viral genes at 6 h p.i. were set as 1 for calculating the fold changes of the mRNAs at other infection time points.

2. Material and methods

2.1. Cells and viruses

Largemouth bass skin (LBS) cells was maintained at 25 °C in DMEM/F-12 medium supplemented with 10% fetal bovine serum (FBS). MSRV was isolated from diseased largemouth bass and propagated in LBS cells. The stocks of MSRV was kept at −80 °C before use.

2.2. Apoptosis detection

To evaluated the cell death induced by MSRV, cells were infected with MSRV at an MOI of 0.1 and then stained with Hochest 33342. Nucleus morphology was observed under fluorescent microscopy.

To examine the caspse-3 activity during MSRV infection, Caspase Colorimetric assay kit (BioVision) was used in this study. In brief, mock- and MSRV-infected cells were harvested at indicated time points (mock, 6, 12, 18, 24, 48 h p.i.) and the spectrophotometric detection of the chromophore *p*-nitroanilide (*p*NA) was carried out according to the manufacture's protocol. The data were expressed as fold increase compared to the corresponding values of caspase activity in lysates of mock-infected cells.

2.3. RNA extraction, cDNA library construction and sequencing

To analyze the expression profiles of host genes involved in MSRV infection, the transcriptome sequencing was performed due to the lack of gene information of largemouth bass. In brief, mock- or MSRV-infected (MOI = 0.1) LBS cells in triplicate flasks were collected and total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer's protocol. After evaluation of the RNA integrity, the libraries were constructed using TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA). Finally, these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500) according to the manufacturer's instructions.