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The innate immune response to grass carp hemorrhagic virus (GCHV) in cultured *Carassius auratus* blastulae (CAB) cells

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Abstract

Virus infection of mammalian cells activates an innate antiviral immune response characterized by production of interferon (IFN) and the subsequent transcriptional upregulation of IFN-stimulated genes (ISGs) by the JAK-STAT signaling pathway. Here, we report that a fish cell line, crucian carp (*Carassius auratus* L.) blastulae embryonic (CAB) cells, can produce IFN activity and then form an antiviral state after infection with UV-inactivated grass carp hemorrhagic virus (GCHV), a double-stranded (ds) RNA virus. From UV-inactivated GCHV-infected CAB cells, 15 pivotal genes were cloned and sequenced, and all of them were shown to be involved in IFN antiviral innate immune response. These IFN system genes include the dsRNA signal sensing factor *TLR3, IFN*, IFN signal transduction factor *STAT*1, IFN regulatory factor *IRF*7, putative IFN antiviral effectors *Mx*1, *Mx*2, *PKR-like*, *Viperin*, *IFI*56, and other IFN stimulated genes (ISGs) *IFI*58, *ISG*15-1, *ISG*15-2, *USP*18, *Gig*1 and *Gig*2. The identified fish IFN system genes were highly induced by active GCHV, UV-inactivated GCHV, CAB IFN or poly(I).poly(C), and showed similar expression patterns to mammals. The data indicate that an IFN antiviral innate immune response similar to that in mammals exists in the UV-inactivated GCHV-infected CAB cells, and the IFN response contributes to the formation of an antiviral state probably through JAK-STAT signaling pathway. This study provides strong evidence for existence of IFN antiviral innate immune response in fish, and will assist in elucidating the origin and evolution of vertebrate IFN system.

Keywords: Interferon response; Interferon system genes; Gene expression; *Carassius auratus* blastulae embryonic (CAB) cells; Grass carp hemorrhagic virus (GCHV)

Abbreviations: IFN, interferon; STAT1, signal transducer and activator of transcription 1; Mx, myxovirus-resistance protein; PKR, dsRNA-dependent protein kinase; IRF, interferon regulatory factor; ISG, interferon stimulated genes, IFI, interferon-inducible; TLR3, toll-like receptor 3; RIG-I, retinoic acid inducible gene I; Mda5, melanoma differentiation-associated gene 5; GCHV, grass carp hemorrhagic virus; CAB, Crucian carp (*Carassius auratus* L.) blastulae embryonic cells; CIK, grass carp (*Ctenopharyngodon idellus*) kidney cells; FCS, fetal calf serum; EST, expressed sequence tag

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1. Introduction

Virus infection of mammalian cells results in the activation of an innate immune response characterized by the production of alpha/beta interferon (IFNα/β) and the subsequent transcriptional upregulation of IFN-stimulated genes (ISGs) [1–3]. In response to virus infection, host cells can rapidly recognize viral pathogens by specific receptors [4]. Toll-like receptor 3 (TLR3), one member of TLR family, was found to recognize extracellular dsRNA in the surrounding medium [4–6]. In addition, the cytoplasmic RNA helicase RIG-I (retinoic acid inducible gene I, also known as Ddx58) and Mda5 (melanoma differentiation-associated gene 5, also known as Ifih 1 and Helicard) were found to recognize intracellular viral dsRNA [7,8]. Upon binding, these receptors activate a variety of signaling pathways leading to the induction of numerous target genes, including IFNα/β [2,4]. Secreted IFNs then participate in an autocrine/paracrine loop by the JAK-STAT pathway that transmits the signal to the nucleus, and results in the expression of a set of ISGs, such as Mx, dsRNA-dependent protein kinase R (PKR) and 2-5 oligo(A) synthetase (2-5 OAS) to inhibit virus replication in host cells [1]. Recent studies showed that other ISGs, including IFI56 [9], IFI54 [10], Viperin (virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible) [11], ISG20 [12], ISG15 [13] and RNA-specific adenosine deaminase (ADAR) [1] also contribute to limit virus replication and spread.

Fish have been used as organisms to study development of the immune system [14,15]. Following virus infection, IFN-like activity can be detected in many fish organs or fish cells. Some genes, such as *IRF*1, *IRF*2 and *IRF*7 [16,17], *Mx* [18,19], *ISG*15 [20], *vig*-1 [21] and *vig*-2 [22], *STAT*1 [23], *JAK*1 [24], and type I *IFN* [25–28], have been cloned and characterized from various fish species, but the information about fish IFN induced genes and their antiviral mechanisms are poorly understood. Owing to the diversity and complexity of the fish innate antiviral immune response, it is worthwhile to monitor the response of fish cells to defined viral pathogens in order to gain specific insights into the nature of the host pathways that govern viral pathogenesis and replication.

Grass carp hemorrhagic virus (GCHV), also called grass carp reovirus (GCRV), belongs to the family *Reoviridae*, genus *Aquareovirus*, and is a dsRNA virus that causes hemorrhagic disease with severe mortality [29]. Once infected, grass carp [30] and their cultured cells [31] secrete an antiviral material. In comparison with active GCHV, UV-inactivated GCHV more effectively induces the antiviral material from cultured cyprinid cell lines, such as crucian carp (*Carassius auratus* L.) blastulae embryonic (CAB) cells [32]. And, the antiviral material has been demonstrated to be indeed crucian carp IFN, since it displays the same antiviral activity as mammalian type I IFN, and the antiviral ability is dependent on protein synthesis and specific to species [33]. Recently, UV-inactivated GCHV infection of CAB cells have been used as an stimulation model for identification and isolation of virally induced genes, and 272 differentially expressed ESTs have been retrieved by suppressive subtractive hybridization technology [34]. So far, several IFN induced genes have been cloned and characterized from this model system [17,19,35–38].

In this study, we confirmed that most of the genes induced by exposure to UV-inactivated GCHV were also induced by treatment with CAB IFN, and identified and characterized pivotal genes of the antiviral innate immune response. Following induction by UV-inactivated GCHV, CAB IFN, and poly(I).poly(C), significant difference in the transcriptional upregulation of these genes were revealed. These results provided evidence for activation of an innate antiviral response in CAB cells when infected with GCHV, and defined some components involved in IFN response in fish.

2. Materials and methods

2.1. Cells and virus

CAB, grass carp (*Ctenopharyngodon idellus*) kidney cells (CIK), grass carp (*C. idellus*) blastulae cells (GCB) and Chinese rare minnow (*Gobiocypris rarus*) embryonic cells (GRE), were grown as monolayers at 28 °C in medium 199 supplemented with 10% fetal calf serum (FCS, heated at 56 °C for 30 min before use), 100 U/ml of penicillin, and 100 μg/ml of streptomycin sulfate. GCHV propagation and UV inactivation were performed according to the previous reports [17,36]. Typically, GCHV in a volume of 3.5–4 ml (1×10^9 $TCID_{50}$/ml) in a 40-mm petri dish was placed on a constantly and slowly shaking shaker, to be UV inactivated with a 30 W general electric germicidal lamp placed at