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Cloning and analysis of antiviral activity of a barramundi (*Lates calcarifer*) Mx gene☆

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Abstract

We obtained a full-length cDNA clone for the Mx gene of barramundi (*Lates calcarifer*), using RACE (rapid amplification of cDNA ends) polymerase chain reaction (PCR) amplification of RNA extracted from a barramundi brain cell line cBB. The Mx cDNA of 2.2 kb contains an open reading frame (ORF) of 1875 nucleotides encoding a protein of 624 amino acids. The predicted barramundi Mx protein is 71.4 kDa and contains a tripartite guanosinetriphosphate (GTP)-binding motif at the amino terminal and a leucine zipper at the carboxyl terminal, characteristic of all known Mx proteins. Poly I:C-transfection induced the expression of Mx gene in cBB cells, and the induction level at 28 °C was higher than that at 20 °C. Moreover, Mx gene expression was also induced by viral infection, including fish nodavirus, birnavirus, and iridovirus. Among these, nodavirus was a stronger inducer than the other two viruses. Using an antiviral activity assay, we revealed that poly I:C-transfected cBB cells had antiviral activity against fish nodavirus and birnavirus, but not iridovirus. Furthermore, the replication of nodavirus and birnavirus could be restored after the expression of Mx gene was down-regulated by siRNA. Therefore, these results indicated that the expression of barramundi Mx gene was able to inhibit the proliferation of fish nodavirus and birnavirus.

Keywords: Mx; Barramundi; cBB cell line; Poly I:C; Nodavirus; Birnavirus; Iridovirus

1. Introduction

The type I interferon (IFN) system is one of the most important mechanisms for antiviral defense. In the type I IFN response, many IFN-related proteins are induced for establishing an antiviral state, such as double-stranded RNA-activated protein kinase (PKR), the 2′, 5′-oligoadenylate synthetase, and Mx proteins [1]. Mx proteins belong to the dynamin superfamily [2] and contain a tripartite GTP-binding domain essential for the antiviral activity [3]. The first Mx protein was found in mice and named because of its resistance to orthomyxovirus influenza A [4]. Mx genes were subsequently found in higher vertebrates, including human [5], some livestock species [6–8], and birds [9,10]. Moreover, some Mx proteins have been shown to inhibit virus replication [11–14].

☆ Statement: The corresponding author (Dr. Chi S.C.) has signed permission from another author (Wu Y.C.) to act on their behalf.

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The lack of information on fish IFN and the difficulty of IFN detection due to its small production and short half-life, led to the use of Mx gene expression as an indicator of IFN activity. Until now, Mx remains useful as a sensitive and more stable marker of the IFN response. In fish, the first Mx gene was identified from perch (*Perca fluviatilis* L.) [15]. Following, piscine Mx genes have been cloned and characterized in rainbow trout (*Oncorhynchus mykiss* Walbaum) [16], Atlantic salmon (*Salmo salar* L.) [17], Atlantic halibut (*Hippoglossus hippoglossus* L.) [18], Japanese flounder (*Paralichthys olivaceus*) [19], fugu (*Takifugu rubripes*) [20], gilthead sea bream (*Sparus aurata*) [21], channel catfish (*Ictalurus punctatus*) [22], and orange-spotted grouper (*Epinephelus coioides*) [23]. However, only few papers have demonstrated the role of fish Mx proteins in IFN response. For example, the Japanese flounder Mx can inhibit the replication of hirame rhabdovirus and viral hemorrhagic septicemia virus (VHSV) [24], while the Atlantic salmon Mx1 can inhibit the replication of infectious pancreatic necrosis virus (IPNV) [25].

Fish nodavirus, nervous necrosis virus (NNV), and fish iridovirus are two major viral pathogens among many species of cultured marine fish in Taiwan [26,27]. The mortality of NNV-infected fish at the larval stage could be as high as 80–100%, and most of the survivors became persistently infected [28]. The information about the mechanism of NNV persistent infection has been very limited until the NNV-persistently infected cell line BB was established [29], and a negative control cell line cBB was obtained by treating BB cells with NNV-specific antiserum [30]. Mx mRNA was detected in the BB cells, but not in the NNV-free cBB cells. In the present study, a barramundi Mx cDNA was cloned from the cBB cells, and the expression of Mx gene in cBB cells was analyzed after poly I:C transfection and virus infection. The antiviral activities against fish nodavirus, birnavirus and iridovirus in poly I:C-transfected cBB cells were examined, and the level of viral replication was further analyzed in cBB cells whose Mx gene expression was down-regulated by siRNA.

2. Materials and methods

2.1. Cell lines and viruses

The cBB cells were cultured with L-15 medium supplemented with 10% fetal bovine serum (FBS), and incubated at 28 °C. Three fish viruses used in the study were: (1) fish nodavirus, B00GD, an NNV isolate of infected barramundi [26]; (2) fish birnavirus, IPNV-SP; and (3) fish iridovirus, TGIV, an isolate from infected grouper [27]. The GF-1 (Grouper Fin-1) cell line [31], the RTG-2 cell line, and the GF-3 (Grouper Fin-3) cell line were respectively used for nodavirus, birnavirus, and iridovirus proliferation and titration.

2.2. Cloning of a barramundi Mx cDNA fragment

A primer set, which was designed from the rainbow trout Mx [16] and channel catfish Mx [22] was chosen to amplify a fragment of the barramundi Mx gene. The sequence of forward primer was 5′-TGAGG AGAAGGTGCGTCC-3′ and that of reverse primer was 5′-CTTCTGACCCCTGCACCTGACGA-3′. The acid guanidinium thiocyanate-phenol-chlorofrom extraction method [32] was used to extract the total RNA from the cBB cells, which were infected with NNV (B00GD) (MOI = 5) for 2 days. Reverse transcription was carried out by incubating 1 μg total RNA at 42 °C for 1 h in 30 μl 1X reaction buffer containing 0.3 μM oligo$(dT)_{20}$, 0.4 mM dNTP, 11.7 mM DTT, 40 U ribonuclease inhibitor rRNasin (Promega), and 60 U MMLV reverse transcriptase (Promega). For PCR reaction, an aliquot (5 μl) of the cDNA was amplified in final volume of 25 μl standard 1X PCR buffer containing 0.4 μM forward and reverse primer, 0.25 mM dNTP, and 0.5 U DyNazyme II DNA polymerase (Finnzymes). The PCR was carried out in GeneAmp PCR System 2400 (Applied Biosystems) with a denaturing step of 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s, with a final extension of 72 °C for 5 min. A 718 bp PCR product was cloned into pGEM-T easy vector (Promega) and sequenced.

2.3. RACE cloning of the full-length barramundi Mx cDNA

RACE was carried out using the Generacer™ primers (Invitrogen), including 5′ RACE Abridged Anchor Primer (AAP), 3′ RACE Adapter Primer (AP) and Abridged Universal Amplification Primer (AUAP). The gene-specific primers (gsp) used in the RACE were designed from the 718 bp fragment sequence of barramundi Mx obtained in Section 2.2, including 5′ RACE first gsp (5′-GCGCCTCCAACACGGAGCTC-3′), 5′ RACE second gsp