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Relationship between Viral Propagation and Apoptosis after Marine Birnavirus (MABV) Infection

Yeong Jin Kim, Won Chul Choi[1], Hyeung Rak Kim[2], Sung Ju Jung, Tae Sung Jung[3], Jae Ho Kim[4], In Kyu Yeo[5] and Myung Joo Oh*

Department of Fish Pathology, Yosu National University, Yosu 550-749, Korea
[1]Department of Biology, Pusan National University, Pusan 609-735, Korea
[2]Department of Food Science & Biotechnology, Pukyung National University, Pusan 608-737, Korea
[3]Department of Microbiology, College of Medicine, Gyeongsang National University, Chinju 660-751, Korea
[4]Graduate School of Biotechnology. Korea University, Seoul 136-701, Korea
[5]Department of Marine Biotechnology, Cheju National University, Cheju 690-756, Korea

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This study was performed to confirm the relationship between viral propagation and apoptosis by the infection of marine birnavirus strain (MABV NF-4) on chinook salmon embryo (CHSE-214) cells. After 6 hr viral infection, MABV was detected by PCR method. Also, as a result of DNA assay on the cells, MABV infection resulted in a typical feature of apoptosis, DNA fragmentation. The results suggest that MABV replicated to high concentrations during the early stage of infection induces apoptosis.

Key words: apoptosis, fish cell line, MABV, birnavirus

Introduction

Cell death can occur by either of two distinct mechanisms, necrosis or apoptosis (Kerr & Harmon 1991). Necrosis is the pathological process which occurs when cells are exposed to a serious physical or chemical insult. Apoptosis is considered to be a physiological process involved in normal tissue turnover, which occurs during embryogenesis, aging, and turnover regression. There are many observable morphological and biochemical differences between necrosis and apoptosis.

Interaction between viruses and their hosts often results in the death of infected cells. Sometimes, viruses may benefit from such a cytopathic reaction; for example, it may facilitate the exit of virus progeny from cells. On the other hand, premature death of the host results in abortive curtailment of the reproductive cycle. Therefore, the time course of the host's death should be controlled. Although viruses may develop specific tools for killing their hosts, they also may exploit preexisting cellular mechanisms. Recent studies have shown that apoptosis may play an important role in many viral infection (Bjorklund et al., 1997). Especially, it was reported that infectious pancreatic necrosis virus (IPNV) causes apoptosis in chinook salmon embryo (CHSE-214) cell lines during the early stages of virus replication, which is then followed by post-apoptotic necrosis (Hong et al., 1998).

Marine birnavirus (MABV), member of the Birnaviridae such as IPNV, IBDV (Brown 1986), causes an acute, contagious disease in number of economically important fish species (Pilcher & Fryer, 1980; Oh et al., 1999a, b). In previously studies, polymerase chain reaction (PCR) is a technique amplifying a specific DNA segment, which is applicate for early diagnosis of many viral disease.

In this study, therefore, we investigated the relationship between virus propagation and cell death in MABV NF-4 (Oh et al., 1999a) infected cell using PCR method and DNA fragmentation assay.

*To whom correspondence should be addressed.
Phone and Fax: 82-61-640-6272
E-mail: ohmj@yosu.yosu.ac.kr.

Materials and Methods

Cell and virus

Chinook salmon embryo cells (CHSE-214) were grown at 18 ℃ in plastic tissue culture flasks (Nunc, Denmark) using Dulbecco's modified Eagle's minimum essential medium (DMEM, GIBCO. BRL) supplemented with 10 % (v/v) fetal bovine serum (FBS, GIBCO. BRL). The infectious MABV NF-4 titer was determined according to the method by Oh et al. (1999b), and the 50 % tissue culture infected dose ($TCID_{50}$) was estimated to 10 $m\ell^{-1}$. The infected cell groups were incubated at 18℃.

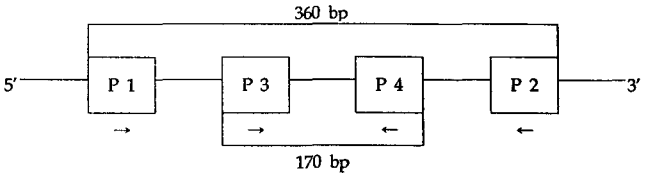
Agarose gel analysis of DNA fragmentation

For DNA fragmentation studies, the infected cells were harvested and rinsed with PBS, then lysed with lysis buffer (10 mM Tris-HCl, 0.25 % Triton X-100, 1 mM EDTA, pH 8.0). Total DNA was recovered by the phenol-chloroform-isoamyl alcohol (25 : 24 : 1) treatment followed by ethanol precipitation, and resuspend in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing DNase-free pancreatic RNase (20 $\mu g/m\ell$). Isolated DNA was electrophoresed in 1.5 % agarose gel for 2 h at 50 V. Gel was stained with ethidium bromide (0.5 $\mu g/m\ell$) and photographed under UV transillumination.

Detection of MABV by PCR amplification

Template RNA was extracted from MABV-infected cells using RNA isolation kit (Boehringer mannheim). cDNA synthesis was performed as described by Suzuki et al. (1997). The two sets of primer, designed from segment A sequences of IPNV Jasper strain, used in this experiment (Suzuki et al., 1997; Fig. 1).

PCR amplifications were performed in 20 $\mu\ell$ of a reaction mixture containing 40 mM KCl, 10 mM Tris-HCl (pH 9.0), 2.5 mM $MgCl_2$, 200 μM of each dNTPs, 100 pM of each primer, 10 ng of template cDNA, and 1 unit of Taq DNA polymerase. The amplification was performed in a thermocycler (Geneamp 2400, Perkin-Elmer) for 1 cycle of 94 ℃ for 5 min, and then 30 cycles of 94 ℃ for 30 sec, 54 ℃ for 1 min, 72 ℃ for 1 min; plus a final 5 min extension at 72 ℃ after 30 cycles. Also, nested PCR was performed as the same condition.



MABV-P1 (25MER): 5'-AGA-GATCAC-TGA-CTT-CAC-AAG-TGA-C-3'
MABV-P2 (24MER): 5'-TGT-GCA-CCA-CAG-GAA-AGA-TGA-CTC-3'
MABV-P3 (17MER): 5'-CAA-CAC-TCT-TCC-CCA-TG-3'
MABV-P4 (17MER): 5'-AGA-ACC-TCC-CAG-TGT-CT-3'

Fig. 1. Schematic representation of the PCR layout used to amplify MABV cDNA, and sequences of the four primers used in the PCR's.

The PCR products were analyzed in 1.5 % agarose gels containing ethidium bromide (0.5 $\mu g/m\ell$), and visualized on UV transilluminator.

Result and Discussion

Cytopathic effect (CPE) appeared at 24 hr after infection by MABV NF-4 (data not suggested). However, DNA pattern of the cell after infection was identified every 6 hr and there was a difference in DNA pattern around 12 hr after infection. And the specific pattern of DNA fragment from 18 hr after infection was shown clear, and that from 24 hr infection showed smeared DNA band (Fig. 2). DNA fragment was not detected in the uninfected cell, which suggests that DNA fragment was caused by viral infection.

On the other hand, a specific PCR product appeared from 6 hr after infection by RT-PCR using MABV-specific primer set (Fig. 3). At this time, non-specific band was detected together. But, non-specific band was not detected in the virus from infected fish. Futhermore, the intensity of a specific DNA band was increase with incubation time, however, that of a non-specific DNA band was decreased with incubation time. This result suggested that the non-specific band was related with DNA from cell was decreased by increment of viral replication.

As previously reported, MABV virions mature within 5 hr after infection and it takes 16~20 hr for a replication (Malsberger and Cerini, 1963). Also, it has been reported for MABV that release of

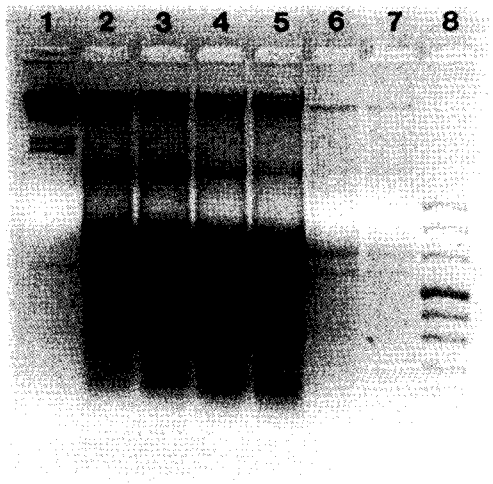


Fig. 2. Agarose gel electrophoresis patterns of the DNA extracted from CHSE-214 cell infected with MABV NF-4 strain. Lanes; 1, λ-Hind III marker; 2, 6 h after infection; 3, 12 h after infection; 4, 18 h after infection; 5, 24 h after infection; 6, 24 h after infection contained 10μg/mℓ CHX; 7, uninfected cell; 8, 100-bp DNA ladder.

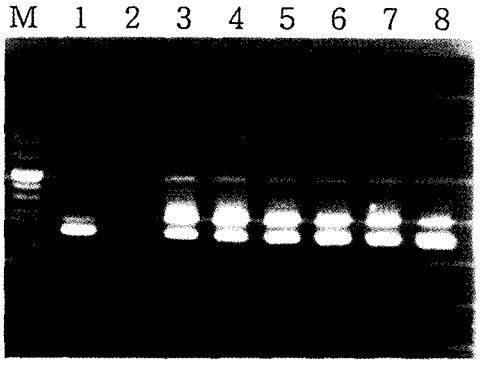


Fig. 3. PCR products in nested PCR. Lanes ; M, PCR marker; 1, the virus from infected fish; 2, uninfected cell; 3, 6 h after infection; 4, 12 h after infection; 5, 18 h after infection; 6, 24 h after infection; 7, 30 h after infection; 8, 36 h after infection.

progeny virus begins approximately 10 hr after infection (Suzuki et al., 1995). Recently, there was reported that infectious pancreatic necrosis virus (IPNV) causes apoptosis in chinook salmon embryo (CHSE-214) cell lines during the early stages of virus replication (Hong et al., 1998) and VP2 protein of infectious bursal disease virus (IBDV), belongs to the same family, is working as an apoptosis inducer (Arias et al., 1997).

Therefore, the above results indirectly suggested that the cell infected by MABV NF-4 caused an apoptosis followed by necrosis and eventually resulted in cell death, and that apoptosis is an important pathogenic mechanism of fish viruses similar to other animal viruses.

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