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Apoptosis in CHSE-214 Chinook Salmon Embryo Cells Infected with Hirame Rhabdovirus (HIRRV)

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In this study, we investigated the mechanism of cell death in rhabdovirus-infected cells, chinook salmon embryonic cell line (CHSE-214) infected with hirame rhabdovirus (HIRRV). Studies using light microscopy, fluorescence microscopy, TUNEL method, electron microscopy, and agarose gel electrophoresis revealed changes in the cell morphology and DNA fragmentation indicative of apoptosis in early infection. It was observed that HIRRV induced apoptosis as well as necrosis in infected cells.

Key words: Apoptosis, Hirame rhabdovirus, HIRRV, CHSE-214 cell

Apoptosis and necrosis are the two morphologically and biochemically distinct modes of cell death in eukaryotic cells (Kerr and Harmon, 1991). Apoptosis is characterized morphologically by cell shrinkage and hyperchromatic nuclear fragments, and biochemically by chromatin cleavage into nucleosomal oligomeres (Wyllie, 1980). Apoptotic cells are subsequently broken down into a number of apoptotic bodies, which are phagocytosed and degraded by the phagocytes.

Apoptosis in fish pathogenic viruses plays a major role in viral propagation, as it has been proved that many animal viruses evade or delay early apoptosis, and actively induce apoptosis in the later stages of infection that would help the virus to produce a high yield of viral progeny (Bjorklund et al., 1997; Hong et al., 1998; Imajoh and Suzuki, 1999; Kim et al., 2000). Hirame rhabdovirus (HIRRV), an RNA virus, was isolated and reported recently from Japanese flounder, *Paralichthys olivaceus* (Oh and Choi, 1998). In the present study, we investigated the mechanisms of cell death in CHSE-214 cells infected with HIRRV.

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Materials and Methods

Virus and Cell line

Hirame rhabdovirus (HIRRV), isolated from diseased flounder from southern coastal area of Korea, was used in this study. The cells used were chinook salmon embryo cells (CHSE-214) cultured in Eagles minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine. HIRRV was inoculated into the cells at 0.1 multiplicity of infection (m.o.i).

Flourescence microscopy

To observe the morphological change, HIRRV-inoculated cells were cultured on chamber slides (Lap Tek, Nunc.) and collected at time intervals of 12 hrs. The collected cells were stained with a mixture (1:1) of acridine orange (270 μM) and ethidium bromide (254 μM) and observed under a fluorescence microscope (OLYMPUS).

TUNEL method

Cells cultured on chamber slide were subjected to TUNEL method. Every 6 hrs after infection, the cells were collected, fixed in 4% paraformaldehyde. The cells were washed with PBS, and subsequently kept in methanol containing 0.3% hydroperoxide. After the pre-treatment, cells were washed with PBS, treated with permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) on ice, again washed PBS, and then dried at room temperature. 50 μL TUNEL reaction mixture was added to the sample for development of color of the infected cells. The samples were put aside in a 37℃, washed in PBS, treated with 50 μL converter-POD solution and then PBS. The samples were further treated with 100 μL DAB-substrate solution and then washed PBS. After a cover glass was mounted on the sample slide, observed under a light microscope (Rojo and Gonzalez, 1998).

Elecron microscopy

At periodic intervals of time, virus-infected cells were collected, fixed in 4% glutaraldehyde and processed for electron microscopic observation. Sections were taken on an ultramicrotome (MTX, RMC Co., USA). The ultra-thin sections were stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (HITACHI-7200, Japan).

DNA fragmentation assay

DNA was isolated from virus-infected cells harvested at periodic intervals of time using apoptotic DNA ladder kit (Roche, Germany). The eluted DNA was precipitated with 0.1 volume of 3 M sodium acetate and 2.2 volumes of ethanol, and resuspended in TE buffer. The purified DNA was electrophoresed in a 1.5% agarose gel. The gel was stained with ethidium bromide (0.5 mg/mL) and photographed using an image analyzer (Fuji, Japan).

Results and Discussion

HIRRV-induced cytopathic effect (CPE) appeared focal at 24 hrs post-infection in CHSE-214 cells. The CPE was subsequently observed throughout the entire monolayer at 36 hrs post-infection (Fig. 1). When compared to the normal uninfected cells, HIRRV-infected CHSE-214 cells at 12 hrs post-infection did not observed any morphological change under fluorescent microscope. The nuclei and cytoplasm appeared green in color (Fig. 2ⓐ, ⓑ). However, 24 hrs after infection, the nuclei and cytoplasm showed weak green color. The color change was more apparent at 36 hrs post-infection. At this stage, cytoplasm appeared bright red-orange and the nuclei turned orange in color, indicating changes similar to apoptotic phenomenon (Fig. 2ⓒ, ⓓ).

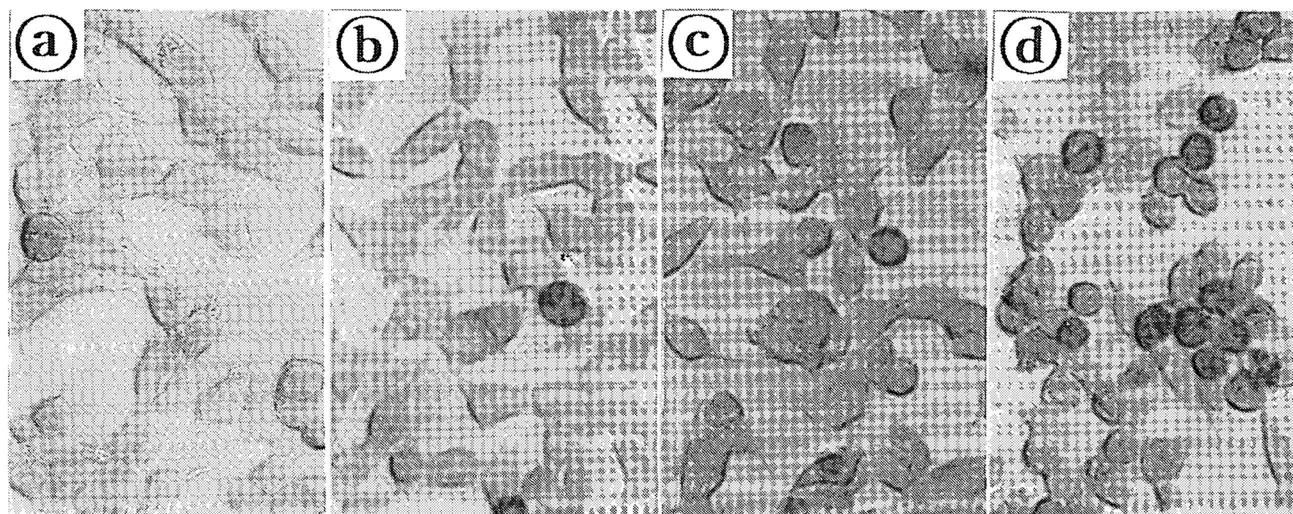

Fig. 1. Morphological changes observed at 12 h intervals after HIRRV infection in CHSE-214 cell under microscope. ⓐ, uninfected control; ⓑ, 12 h post-infection; ⓒ, 24 h post-infection; ⓓ, 36 h post-infection.

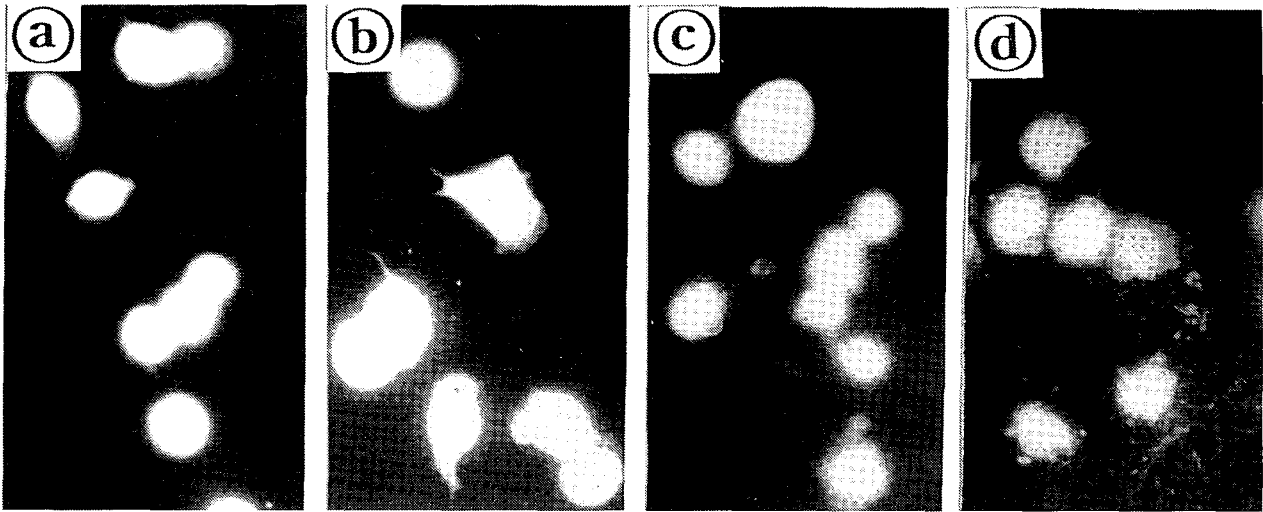

Fig. 2. Morphological changes of apoptotic cell death at 12 h intervals after HIRRV in CHSE-214 cell by TUNEL method. ⓐ, uninfected control; ⓑ, 12 h post-infection; ⓒ, 24 h post-infec-tion; ⓓ, 36 h post-infection.

Morphological changes such as cytoplasmic and nuclear condensation were detectable at 36 hrs post-infection. However, weak green color was found persisting at this stage. The cells in the late apoptotic stages could be identified due to their orange color and necrotic cells with their bright orange nuclei and dark red cytoplasm. Therefore, not only the cell morphology but also the appearance of dark red nuclei among virus-infected cells suffering cell death can be the evidence of apoptosis (Rojoand Gonzalez, 1998). Although there was some difference in the time interval of cell damage in the cells cultured in chamber slides compared to the cells under normal culture condition, the general features of cell death followed similar pattern.

The observation of infected cells with transmission electron microscope did not found any signs of apoptotic changes until the 6 days post-infection. However, cells at 6 days post-infection revealed typical characteristics of apoptosis such as nuclear fragmentation and cytoplasmic condensation (Fig. 3). DNA fragmentation assay showed no DNA ladder until 3 days post-infection, when compared to uninfected cells. However, weak laddering was observed at 4 days of post-infection. Five days post-infection, apparent laddering of 200 bp DNA fragment appeared but the bands were found to smear in the gel after 6 days of infection. The smearing degree of the bands was found to be increased as the infection progressed (Fig. 4). This

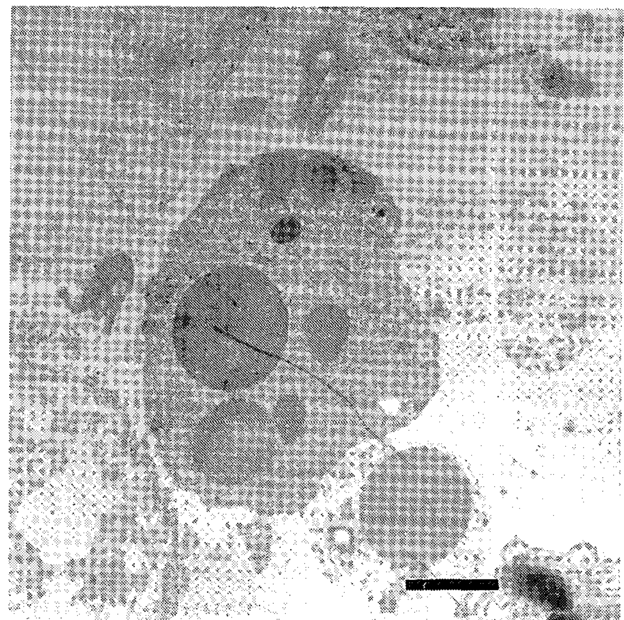

Fig. 3. HIRRV-infected cell at 144 h post-infection under electron microscope (Showing the chromatin condensation and apoptotic bodies in the cytoplasm) Bar= 1 μm.

indicates that apoptosis and necrosis are consecutive phenomena in the viral infection depending on the fact that apoptotic DNA and necrotic DNA appear in a ladder pattern and a smear pattern, respectively (Peitsch et al., 1993; Janicke et al., 1998). Therefore, the present study indicates that HIRRV can induce apoptosis in CHSE-214 cells. And, there are clear evidences to suggest that the mechanism

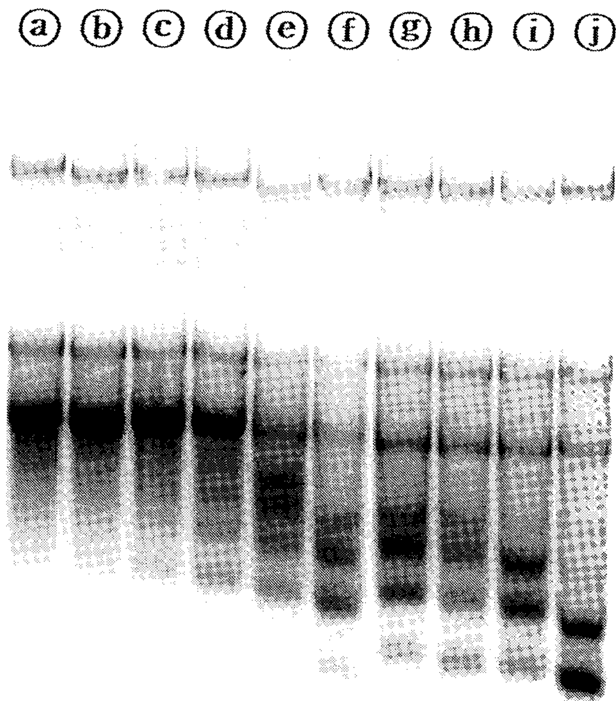

Fig. 4. Agarose gel electrophoresis pattern of the DNA isolated from HIRRV-infected CHSE-214 cells at different times after the infection. ⓐ, control; ⓑ, 48 h; ⓒ, 60 h; ⓓ, 72 h; ⓔ, 84 h; ⓕ, 96 h; ⓖ, 108 h; ⓗ, 120 h; ⓘ, 132 h; ⓙ, 144 h.

of cell death in HIRRV infection is by an early-stage apoptosis and late post-apoptotic necrosis. This is in accordance with the hypothesis made by Hong et al. (1998) in the case of IPNV infection in CHSE-214 cells. The pattern of cell death caused by HIRRV also showed resemblance to MABV-induced apoptosis in fish cell lines reported by Imajoh and Suzuki (1999).

References

Bjorklund, H.V., T.R. Johansson and A. Rinne. 1997. Rhabdovirus-induced apoptosis in a fish cell line is inhibited by a human endogenous acid cysteine proteinase inhibitor. J. Virol., 71, 5658~5662.

Hong, J.R., T.L. Lin, Y.L. Hsu and J.L. Wu. 1998. Induction of apoptosis and secondary necrosis by infectious pancreatic necrosis virus in fish embryonic cells, J. Fish Dis., 2, 463~467.

Imajoh, M. and S. Suzuki. 1999. Apoptosis induced by a marine birnavirus in Established cell lines from fish. J. Fish Pathol., 34, 73~79.

Janicke, R.U., M.L. Sprengart, M.R. Wati and A.G. Porter. 1998. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J. Biol. Chem., 273, 9357~9360.

Kerr, J.F.R. and B.V. Harmon. 1991. Definition and incidence of apoptosis: an historical perspective. In: Apoptosis: The molecular basis of cell death L.D. Tomoei & F.O. Cope, ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor. NY, pp. 5~29.

Kim, Y.J., W.C. Choi, H.R. Kim, S.J. Jung, T.S. Jung, J.H. Kim, I.K. Yeo and M.J. Oh. 2000. Relationship between viral propagation and apoptosis after marine birnavirus (MABV) infection. J. Fish. Sci. Tech., 3, 49~51.

Oh, M.J. and T.J. Choi. 1999. A new rhabdovirus (HRV-like) isolated in Korea from cultured Japanese flounder *Paralichthys olivaceus.* J. Fish Pathol., 11, 129~136.

Peitsch, M.C., C. Muller and J. Tschopp. 1993. DNA fragmentation during apoptosis is caused by frequent single-strand cuts. Nucleic Acids Res., 21, 4206~4209.

Rojo, M.C. and M.E. Gonzalez. 1998. *In situ* detection of apoptotic cell by TUNEL in the gill epithelium of the developing brown trout (*Salmo trutta*). J. Anat., 193, 391~398.

Wyllie, A.H. 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogeneous endonuclease activation. Nature, 284, 555~556.