Differential apoptosis regulation by RSIV and ISKNV in DGF cells

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Introduction

Megalocytivirus, including red sea bream iridovirus (RSIV) and infectious spleen and kidney necrosis virus (ISKNV), is a major viral pathogen that causes severe disease in marine and freshwater fish species. Apoptosis is a critical host defense mechanism against viral infection, regulating cell death to limit viral spread. However, the role of apoptosis during RSIV and ISKNV infection at the cellular level remains insufficiently understood. In this study, a dwarf gourami fin (DGF) cell line was utilized to investigate the relationship between RSIV and ISKNV replication and apoptosis regulation, with a focus on cytopathic effects and apoptosis-related responses.

Materials and Methods

1. Cell line and Virus

- DGF cell line was maintained in L-15 medium containing 10% FBS, 1 × gentamicin, MEM non-essential amino acid solution, and 10 mL of HEPES. DGF cells were incubated at 28°C. RSIV (19V strain) and ISKNV (DGIV-SAY-23) used in this study was isolated from diseased rock bream (*Oplegnathus fasciatus*) and diseased dwarf gourami (*Trichogaster lalius*).

2. Cell viability under RSIV and ISKNV infection

- DGF cells were seeded in the 96-well plate at a concentration of 2.5 × 10^5 cells/mL, and viruse at a concentration of $10^{5.8}$ $TCID_{50}$/well was adsorbed onto DGF cells for 2 hours. Cell viability was evaluated at 1-7 dpi using a Cell Counting Kit-8.

3. Viral replication in DGF cell line

- DGF cells were infected with RSIV and ISKNV at a concentration of $10^{5.8}$ $TCID_{50}$/well. After 2 hours, cells were washed twice by PBS containing 1 × gentamicin. Supernatants were collected at 12, 24, 36, 48, 72, 84, and 96 hpi, and viral genome copy numbers were quantified by real-time PCR.

3. Gene expression analysis

- DGF cells were infected with RSIV and ISKNV under same conditions described above. Total RNA was extracted, and cDNA was synthesized. The expression levels of the MCP gene and apoptosis-related genes were analyzed by real-time PCR at 12, 24, 48, 72, 84, and 96 hpi.

4. Apoptosis analysis

- DGF cells were infected with RSIV and ISKNV under the same conditions described above. Apoptosis was evaluated by measuring mitochondrial membrane potential (MMP) loss using JC-1 staining. Samples were collected at 1, 2, 3, and 4 days post-infection (dpi).

5. Cisplatin treatment and viral replication

- DGF cells were infected with RSIV and ISKNV under the same conditions described above. After virus adsorption for 2 h, the inoculum was removed, and the cells were treated with 25 μM of cisplatin. The supernatants were collected at 1, 2, 3, and 4 days post-infection (dpi), and viral genome copy numbers were quantified by real-time PCR.

Results

1. Effect of RSIV and ISKNV infection on cell viability

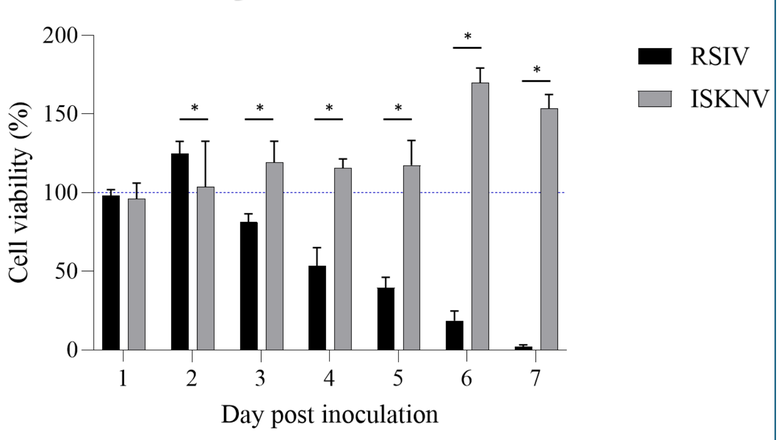


Fig.1 Effect of RSIV and ISKNV infection on cell viability in DGF cells. Cell viability was measured at 1-7 dpi.

- RSIV infection showed progressive decrease in cell viability, while ISKNV infection showed higher viability than mock-infected cells

2. RSIV and ISKNV replication in DGF cell

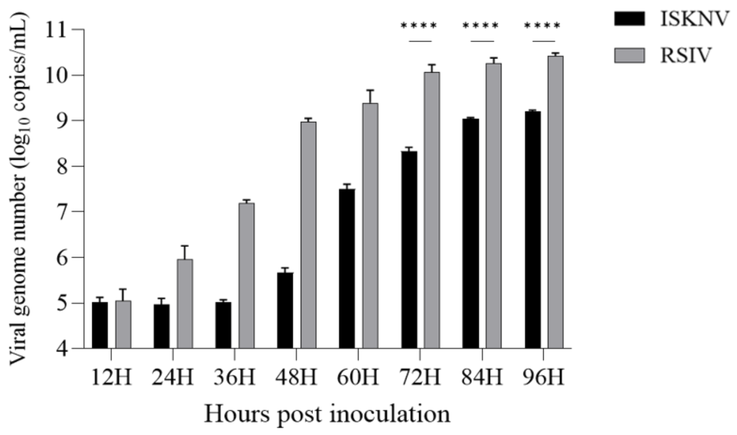


Fig.2 RSIV and ISKNV replication in DGF cells. Viral genome copy numbers (log_{10} copies/mL) were quantified at 12–96 h post-infection (hpi) by real-time PCR.

- RSIV showed more rapid and aggressive viral replication than ISKNV in DGF cell.

3. Apoptosis related gene expression

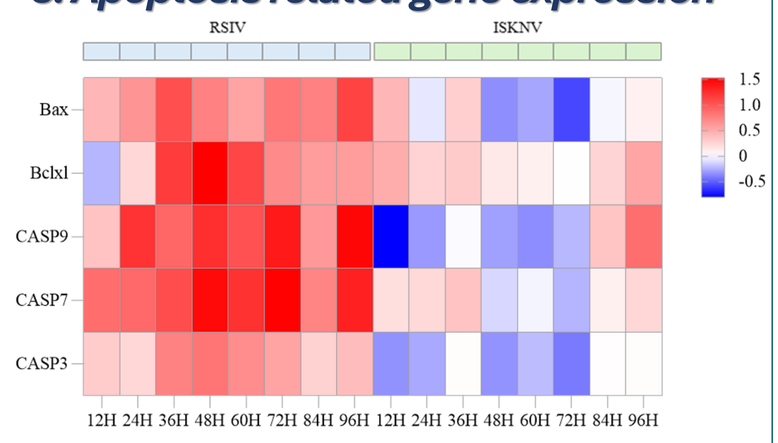


Fig.3 Expression of apoptosis-related genes in RSIV- or ISKNV-infected DGF cells. Relative expression levels of apoptosis-related genes were analyzed by real-time PCR at indicated time points and normalized to β-actin.

- RSIV induced stronger apoptosis-related responses than ISKNV in DGF cells.

4. JC-1 staining on virus infected DGF cell

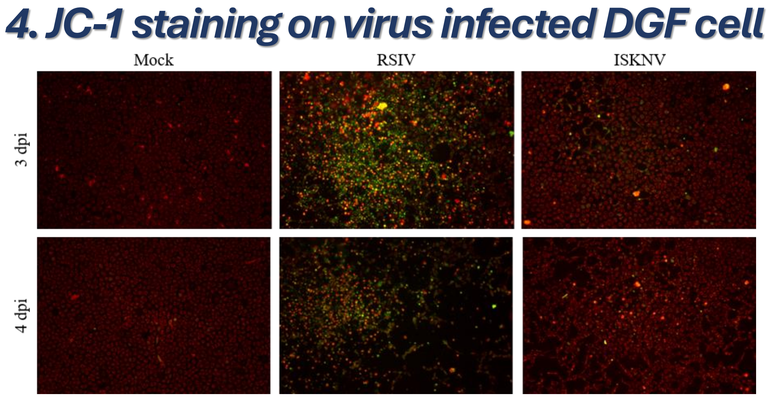


Fig.5 Mitochondrial membrane potential (MMP) was assessed by JC-1 staining in mock-, RSIV- or ISKNV-infected cells. Loss of MMP is indicated by increased green fluorescence.

- RSIV infection showed prominent green fluorescence at 3-4 dpi, leading MMP loss.
- ISKNV infection showed limited green fluorescence, leading less pronounced MMP loss.
- Stronger ΔΨm loss in RSIV than in ISKNV infection.

5. Cisplatin treatment during viral infection

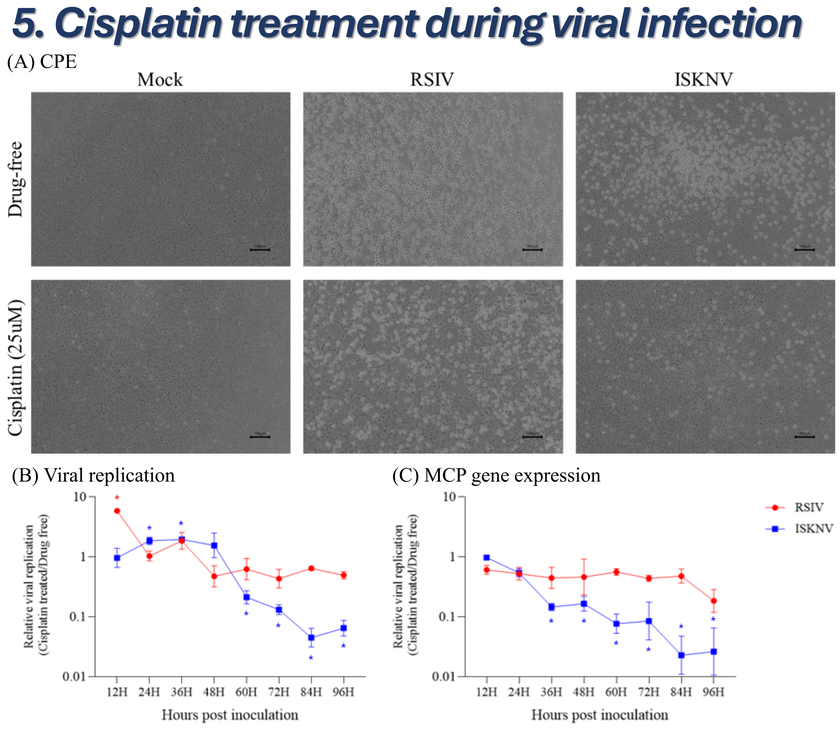


Fig.6 Effect of cisplatin on viral infection in DGF cells. (A) Cell morphology, (B) Relative viral replication after cisplatin treatment.

- Cisplatin treatment reduced number of enlarged cells in RSIV and ISKNV infected DGF cells.
- RSIV replication was largely unaffected by apoptosis induction, while ISKNV replication was significantly inhibited under apoptosis-inducing condition.
- Indicating distinct replication under pro-apoptotic cellular enivronments.

