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Establishment and Characterization of a Fin Cell Line Derived from the Atlantic Salmon *Salmo salar* and Its Application to Fish Virology Study

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Abstract Atlantic salmon (*Salmo salar*) is an important economic fish that is seriously threatened by various viruses. A cell line designated as ASF derived from the caudal fin tissue of Atlantic salmon was established and characterized in this study. ASF cells grew well in Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal bovine serum at 20℃. DNA sequencing and comparative analysis of the *cytochrome B* gene verified that the ASF cell line originated from Atlantic salmon. Chromosome analysis indicated that the modal chromosome number of ASF cells was 58. Viral susceptibility test showed that ASF cells were susceptive to two important fish viruses, viral hemorrhagic septicemia virus (VHSV) and red-spotted grouper nervous necrosis virus (RGNNV). Viral replication in ASF cells was further confirmed by qRT-PCR and transmission electron microscopy. Moreover, VHSV and RGNNV infections could induce the cellular responses in ASF cells, as indicated by the differential expression of cellular antiviral response-related genes, *interferon-1* and *Mx-1*. In conclusion, the newly established ASF cell line can be applied as an *in vitro* tool in fish virology and immunity studies.

Key words *Salmo salar*; cell line; viral hemorrhagic septicemia virus; nervous necrosis virus; immune response

1 Introduction

Atlantic salmon (*Salmo salar*) is a popular aquatic product because of its delicious taste and rich nutrition (Lozano-Muñoz *et al*., 2020). With the decline of wild fish populations due to environmental pollution and habitat destruction, artificial farming has become the main way for people to obtain Atlantic salmon (Jin *et al*., 2020). Atlantic salmon is a significant salmonid species in terms of value and production scale in global aquaculture (Brudeseth *et al*., 2013). However, more and more viruses infect Atlantic salmon (Munro *et al*., 2015; Eriksson-Kallio *et al*., 2020; Gjessing *et al*., 2020; Jenberie *et al*., 2020; Pham *et al*., 2020; Samsing *et al*., 2021). Viral hemorrhagic septicemia virus (VHSV) is a massively destructive virus with a high mortality rate of up to 100% in fry, causing great losses to Atlantic salmon aquaculture (Emmenegger *et al*., 2013; Ito *et al*., 2016; Zhang *et al*., 2019). Nervous necrosis virus (NNV), infecting more than 120 marine and freshwater teleost species, is also considered a potential threat to salmon farming (Li *et al*., 2019; Jia *et al*., 2020; Zhang *et al*., 2020). Although no report stated that NNV infects salmon under natural conditions, Korsnes *et al*. (2005) found that viral nervous necrosis broke out in Atlantic salmon after intraperitoneal challenge with nodavirus from Atlantic halibut (*Hippoglossus hippoglossus*). Therefore, the pathogenic mechanisms of NNV and VHSV in Atlantic salmon must be elucidated to develop an effective therapy.

Fish cell lines are essential tools for culturing viruses, studying the mechanism of host-virus interactions, and developing vaccines (Collet *et al*., 2018; Pham *et al*., 2020). For instance, the FtGF cell line, derived from the fin of fantail goldfish, was used for the *in vitro* propagation of Cyprinid herpes virus-2 (Dharmaratnam *et al*., 2020). Transcriptome analysis was performed in hirame natural embryonic cells with or without NNV infection to determine the innate immune response-related genes against the virus (Kim *et al*., 2020). A formalin-inactivated red sea bream iridovirus vaccine was also developed using the culture supernatant of a *Pagrus major* fin cell line persistently infected with IVS-1 strain (Kwon *et al*., 2020). Although more than 880 fish cell lines have been established, more cell lines from various species are still needed to meet the diverse needs of virology and immunology studies, considering the specificity of host-virus interaction (Robin *et al*.,

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2020). Several cell lines have been established from different tissues of Atlantic salmon (Martin *et al*., 2007; Rodriguez Saint-Jean *et al*., 2014; Pham *et al*., 2017b; Gjessing *et al*., 2018). Some of these cell lines are susceptible to various viruses, including infectious hematopoietic necrosis virus, VHSV, infectious pancreatic necrosis virus, Atlantic salmon reovirus, and Pacific salmon paramyxovirus, and the cell lines have been used to develop antiviral strategies (Gjessing *et al*., 2018; Rodriguez Saint-Jean *et al*., 2014). However, none of them are susceptible to NNV. In the present study, an Atlantic salmon caudal fin-derived cell line that is susceptible to NNV and VHSV was established and characterized. This study can be a basis to elucidate the infection mechanisms of NNV and VHSV in Atlantic salmon and contribute to the AS invitrome, a collection of different cell lines derived from this species (Bols *et al*., 2017).

2 Materials and Methods

2.1 Fish and Viruses

Healthy juvenile Atlantic salmon (approximately 50 g in weight) for the development of primary cells were obtained from a local fish farm in Shangdong Province, China, and were maintained in seawater at 15℃. All procedures carried out with Atlantic salmon were approved by the Ethics Committee of Sun Yat-sen University. The fish were completely anesthetized using MS222 before euthanasia (Sigma, St. Louis, MO).

Red-spotted grouper nervous necrosis virus (RGNNV) (strain SBN147) was isolated from sicked sea perch and propagated in *Lateolabrax japonicus* brain cells (Le *et al*., 2017). VHSV IVa (strain VHSVLB2018) was isolated from largemouth bass and propagated in *Pampus argenteus* fin cells (Zhang *et al*., 2019).

2.2 Primary Cell Culture and Subculture

The work was carried out on a Clean Bench, and sterile techniques were employed in all cell culture procedures. First, the surface of Atlantic salmon was sterilized by 75% ethanol before dissection. Then, caudal fin tissues were detached and transferred to a sterile Petri dish supplemented with 5 mL of phosphate-buffered saline (PBS) containing antibiotics (penicillin, 100 U mL^{-1}; streptomycin, 100 μg mL^{-1}) for washing three times. Then, the fin samples were minced into pieces and transferred into a 25 cm^2 culture flask containing 1 mL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum (FBS) and 4.76 g L^{-1} HEPES with a final pH of 7.4, and then were cultured at 20℃. Subsequently, 3 mL of DMEM was added after the explants adhered to the bottom of the flask. The medium was exchanged every 4−5 days. When cell monolayers had developed, they were detached with DMEM containing 20% FBS using the standard trypsinization method, and subcultured at 1:2 split. The cell culture was denoted as 'ASF cells'.

2.3 Species Authentication

A partial sequence of the Atlantic salmon *cytochrome B* (*Cyt B*) gene was analyzed as previously described with some modifications to identify the origin of ASF cells (Li *et al*., 2019). Total genomic DNAs were extracted from Atlantic salmon caudal fin tissues and ASF cells (at passage 10) by using the Tissue DNA Kit (Omega, Norcross, GA), respectively. A 514 bp fragment of the *Cyt B* gene was amplified using primers *Cyt B*-F and *Cyt B*-R (Supplemented in Table 1). The PCR products were then sequenced and analyzed.

Table 1 Primers used for cloning and expression analysis

Name	Sequence (5'–3')	Comment
Cyt B-F	CACGATTTTTCGCCTTCC	Partial sequence of *Cyt B*
Cyt B-R	AATGGGTGTTCCACGGGT	Partial sequence of *Cyt B*
N-F	ATAAACAAGGGCTTGGTCTC	Partial sequence of *N*
N-R	AGAAGTCGTCTGTGGCTCCC	Partial sequence of *N*
CP-F	TACGCAAAGGTGAGAAGA	Partial sequence of *CP*
CP-R	CACAGGAGTATCAGCCGA	Partial sequence of *CP*
IFN-1F	AAAACTGTTTGATGGGAATATGAAA	QPCR of *IFN-1*
IFN-1R	CGTTTCAGTCTCCTCTCAGGTT	QPCR of *IFN-1*
Mx1-F	AGCTCAAACGCCTGATGAAG	QPCR of *Mx-1*
Mx1-R	ACCCCACTGAAACACACCTG	QPCR of *Mx-1*
RDRP-QPCR-F	CCTCACCAACACTGCTTCTTC	QPCR of *RDRP*
RDRP-QPCR-R	CCAGCCAATGTCGTCAATCTC	QPCR of *RDRP*
G-QPCR-F	AACTGTCTCCAAAGAAGTGTGT	QPCR of *G*
G-QPCR-R	GCCATCAAGGAGATAATGTG	QPCR of *G*
EF-F	GATCCAGAAGGAGGTCACCA	QPCR of *EF-1α*
EF-R	TTACGTTCGACCTTCCATCC	QPCR of *EF-1α*

2.4 Chromosome Analysis

Chromosomes of ASF cells were analyzed at passage 20. Cells were seeded in 25 cm^2 culture flasks and treated with colchicine (Sigma) (10 μg mL^{-1}) at 70%−80% confluence for 24 h. The cells were harvested and centrifuged at 1000 r min^{-1} for 5 min, resuspended in 1 mL of 0.075 mol L^{-1} KCl, and then incubated at 37℃ for 45 min. Then, the cells were fixed with freshly mixed methanol and acetic acid (3:1, V/V) for 10 min and collected by centrifuging at 1000 r min^{-1} for 5 min. After one more fixation, cells were dropped onto pre-cooled microslides and stained with 10%

Giemsa (Sigma). Chromosomes were photographed and counted.

2.5 Growth Curves

For growth characteristic studies, ASF cells (25th passage) were seeded in 24-well plates. Cells were cultured at 10℃, 15℃, 20℃, and 28℃ to determine the effect of temperature on cell growth. Cells were cultured in DMEM containing different concentrations of FBS (10%, 15%, and 20%) to investigate the influence of FBS concentration. Cells were harvested and counted using a hemacytometer after 4, 8, 12, and 14 days, respectively. Experiments were conducted in triplicate.

2.6 Cell Migratory and Proliferative Abilities

Migratory and proliferative abilities of ASF cells (passage 16) were determined as previously described with some modifications (Gjessing *et al*., 2018). ASF cells were seeded in 6-well plates and cultured at 20℃ until confluent monolayers developed. A scratch was made down through the cell monolayer by a sterile 1 mL pipette tip. The medium was changed, and the scratch was photographed daily for 7 days. The width of the scratch was measured in cm on enhanced snapshots.

2.7 Viral Susceptibility and Replication

RGNNV and VHSV were applied to evaluate the viral susceptibility of ASF cells. Cells were seeded into 6-well plates and grown to 80% confluence for infection. Then, the cells were infected with RGNNV or VHSV (multiplicity of infection = 1) at 28℃ and 20℃. After adsorbing for 4 h, the medium was exchanged. ASF cells incubated with PBS served as controls. Cytopathic effects (CPEs) were assessed daily, and the cells were collected at 24 and 48 h post infection (hpi) for RT-PCR and qPCR assays to detect RGNNV and VHSV replication as previously described (Li *et al*., 2019).

Viral replication was confirmed *via* transmission electron microscopy. ASF cells were seeded into a 75 cm^2 culture flask and infected with RGNNV or VHSV as mentioned above. The infected cells were harvested and fixed with 2.5% glutaraldehyde at 4℃ for 24 h and then fixed with 2.0% osmium tetroxide for 1 h. The samples were dehydrated by graded ethanol (30%, 50%, 70%, 80%, 95% and 100%), embedded in epoxy resin for sectioning, and then stained with uranyl acetate/lead citrate. The viral particles were observed under a Philips CM10 electron microscope.

2.8 Expression Analyses of *IFN-1* and *Mx-1* Genes in ASF Cells After RGNNV and VHSV Infections

ASF cells were infected by RGNNV or VHSV as described in 'Viral susceptibility and viral replication'. Cells were harvested for RNA extraction at 24 and 48 hpi, and then cDNAs were synthesized using PrimeScript Reverse Transcriptase in accordance with the manufacturer's instructions. Transcription levels of *interferon-1* (*IFN-1*) and *Mx-1* were determined by qRT-PCR in a LightCycler 480 II (Roche) as previously described (Zhang *et al*., 2018). The Atlantic salmon gene *EF-1α* served as the reference gene. Primers for qRT-PCR are listed in Table 1. All samples were performed in triplicate, and the $2^{-\Delta\Delta Ct}$ method was used to analyze gene expression levels.

2.9 Statistical Analysis

Data represented the average value of three replicates and were expressed as mean ± SD (standard deviation). SPSS version 20 was used for statistical analysis. Statistical differences between different groups were determined using one-way ANOVA. Statistical significance was considered at $P < 0.05$.

3 Results

3.1 Primary Culture and Subculture of ASF Cells

The ASF cell line was derived from the caudal fin of healthy juvenile Atlantic salmon. Three days after seeding, epithelial-like cells began to migrate from the explants. The cells grew to full confluence 15 days later (Fig.1A). Then, the cells were subcultured at a split ratio of 1:2 every 7–10 days in DMEM (15% FBS) at 20℃. Until now, ASF cells have been subcultured more than 50 times (Fig.1B).

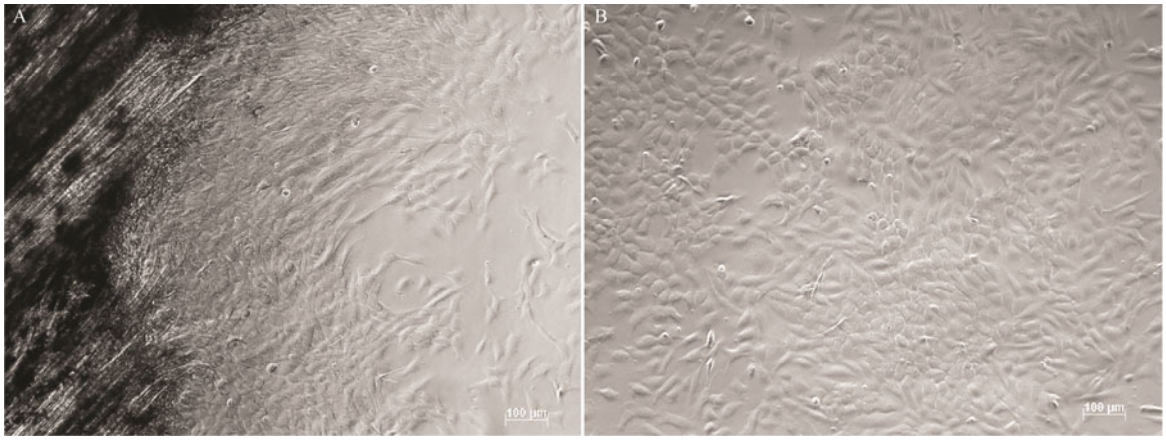

Fig.1 Morphology of cells derived from the caudal fin of Atlantic salmon. A, Primary cultures on day 6; B, ASF cells at the 50th passage. Bar = 100 μm.

3.2 Species Authentication and Karyotyping

Partial amplification of the C*yt B* gene was performed using ASF cells and caudal fin tissue of Atlantic salmon to evaluate the potential origin of ASF cells, and an expected fragment of 514 bp was amplified (Fig.2A). Subsequent sequencing showed that the sequenced fragments from the ASF cells and caudal fin tissue of Atlantic salmon were identical, and revealed 99.6% similarities with the known *Cyt B* sequence of Atlantic salmon (GenBank accession no. JQ390056.1). These results verify that the ASF cell line was derived from Atlantic salmon.

One hundred metaphases plates were analyzed to determine the chromosome numbers of ASF cells at passage 20. As shown in Figs.2B and 2C, the modal chromosome number of ASF cells was 58.

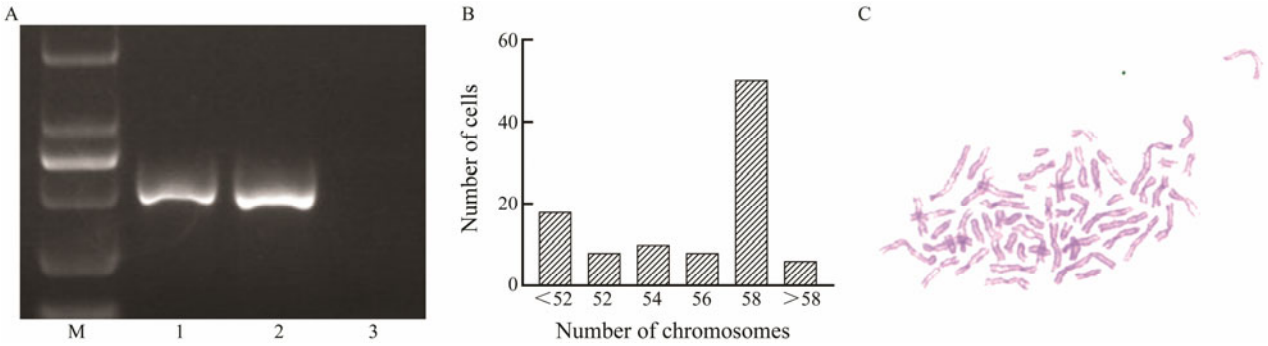

Fig.2 Species authentication and karyotyping. A, PCR amplification of partial *Cyt B* gene sequences in ASF cells and Atlantic salmon fin tissue. M, DNA marker (2000 bp); Lane 1, ASF cells; Lane 2, fin tissue; Lane 3, negative control. B, Frequency distribution of chromosomes in ASF cells counted at the 20th passage. C, Giemsa-stained metaphase chromosomes.

3.3 Cell Growth Characteristics

The effects of temperature and FBS concentration on ASF cell growth at passage 25 were tested. ASF cells grew best in DMEM with 20% FBS, and slowed growth rate was observed with decreasing concentration of FBS at 20℃ (Fig.3A). For temperature, ASF cells cultured in DMEM with 20% FBS exhibited a maximum growth rate at 20℃ (Fig.3B).

3.4 Migratory and Proliferative Abilities

Cell migration assay showed a gradually healing scratch of ASF cells after 7 days (Figs.4A–4F). The width variation of the scratch is shown in Fig.4G.

3.5 Viral Susceptibility and Viral Replication

Compared with the control cells (Figs.5C and 5F), RGNNV- or VHSV-infected ASF cells exhibited significant CPEs. After challenging with RGNNV, ASF cells showed shrinking characteristics and appeared spherical at 24 hpi, and approximately 90% of cells detached from the bottom of the plate at 48 hpi (Figs.5A and 5D). A few ASF cells infected with VHSV started to show morphological changes, such as cell rounding and detachment, at 24 hpi (Fig.5B). Then, the monolayers were damaged gradually and disintegrated at 48 hpi (Fig.5E). Subsequently, infected ASF cells were confirmed by RT-PCR. Partial fragments of the RGNNV *CP* gene and the VHSV *N* gene were amplified from RGNNV- and VHSV-infected ASF cells, respectively (Figs.5G, 5H).

The proliferation of RGNNV and VHSV in ASF cells was further confirmed. The expression of RGNNV *RDRP* and VHSV *G* increased significantly from 24 to 48 hpi in RGNNV- or VHSV-infected ASF cells, respectively (Figs.6A and 6D). Moreover, transmission electron microscopy revealed the presence of several virus particles in the cy- toplasm of RGNNV- or VHSV-infected ASF cells (Figs.6B, 6C and Figs.6E, 6F).

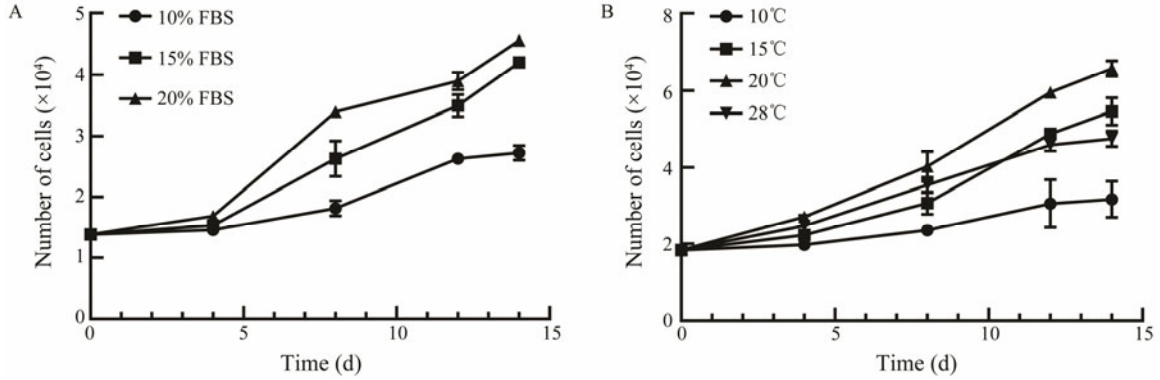

Fig.3 Effects of FBS concentrations and temperatures on the proliferation of ASF cells. A, ASF cells were incubated with DMEM containing 10%, 15%, or 20% FBS at 20℃; B, ASF cells were incubated with DMEM containing 20% FBS at 10℃, 15℃, 20℃, or 28℃.

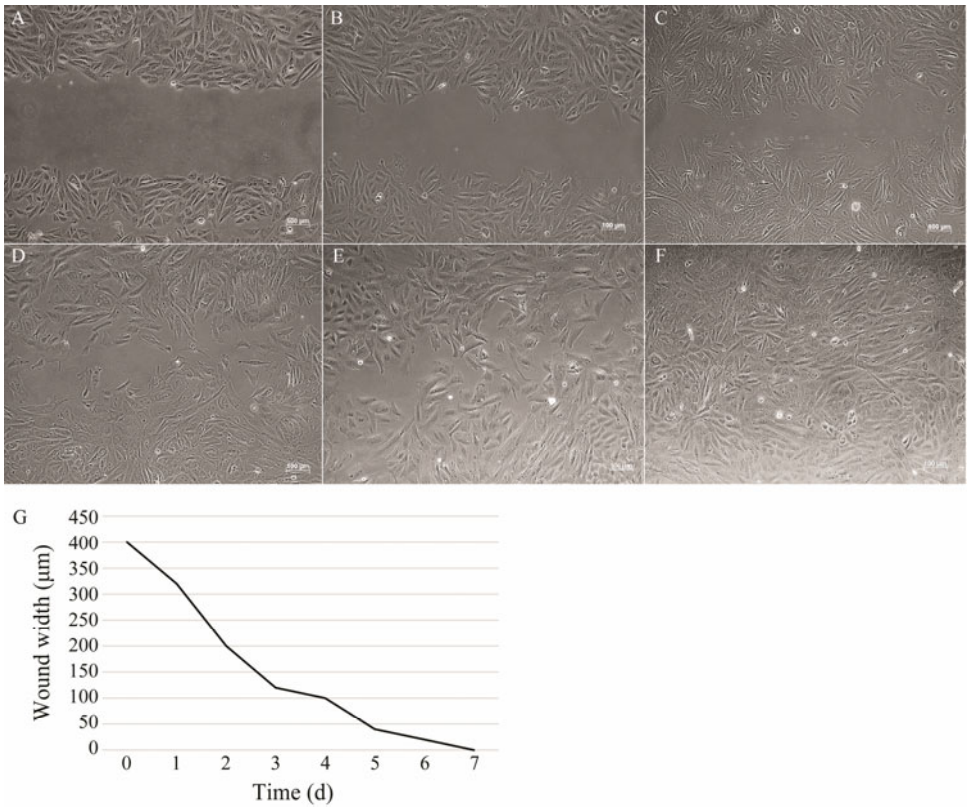

Fig.4 Wound healing. A scratch was made through the ASF cell monolayer by a sterile 1 mL pipette tip. Cells were cultured continuously at 20℃ and photographed every day. Representative pictures of ASF at day 0 (A), day 1 (B), day 2 (C), day 3 (D), day 4 (E), and day 7 (F) are shown. Bar = 100 μm. G, measurement of scratch width.

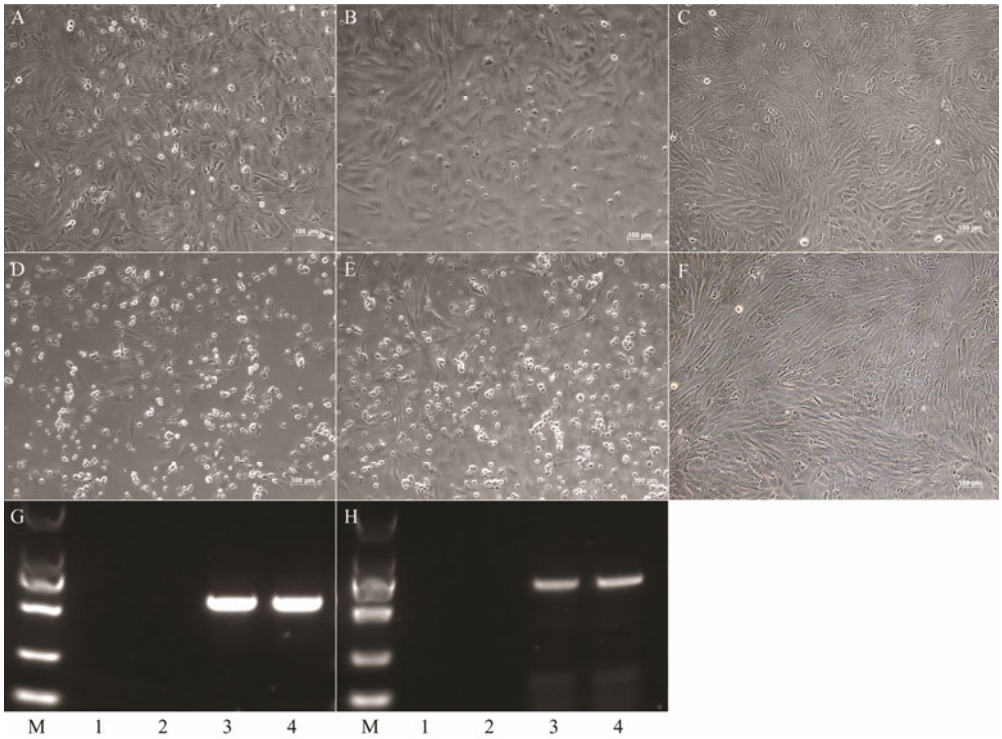

Fig.5 Susceptibility of ASF cells to RGNNV and VHSV. (A), (D) showed CPEs of ASF cells infected with RGNNV at 24 hpi and 48 hpi, respectively. (B), (E) showed CPEs of ASF cells infected with VHSV at 24 hpi and 48 hpi, respectively. (C), (F) showed Mock-infected ASF cells at 24 h and 48 h , respectively. Bar = 100 μm. (G) showed agarose gel electrophoresis of PCR products from RGNNV-infected ASF cells using specific primer for the RGNNV *CP* gene. M, DNA marker (2000 bp); Lane 1, ASF cells without RGNNV infection; Lane 2, blank control; Lane 3, ASF cells infected with RGNNV for 48 h; Lane 4, RGNNV positive control. (H) showed agarose gel electrophoresis of PCR products from VHSV-infected ASF cells using specific primers for the VHSV *N* gene. M, DNA marker (2000 bp); Lane 1, ASF cells without VHSV infection; Lane 2, blank control; Lane 3, ASF cells infected with VHSV for 48 h; Lane 4, VHSV positive control.

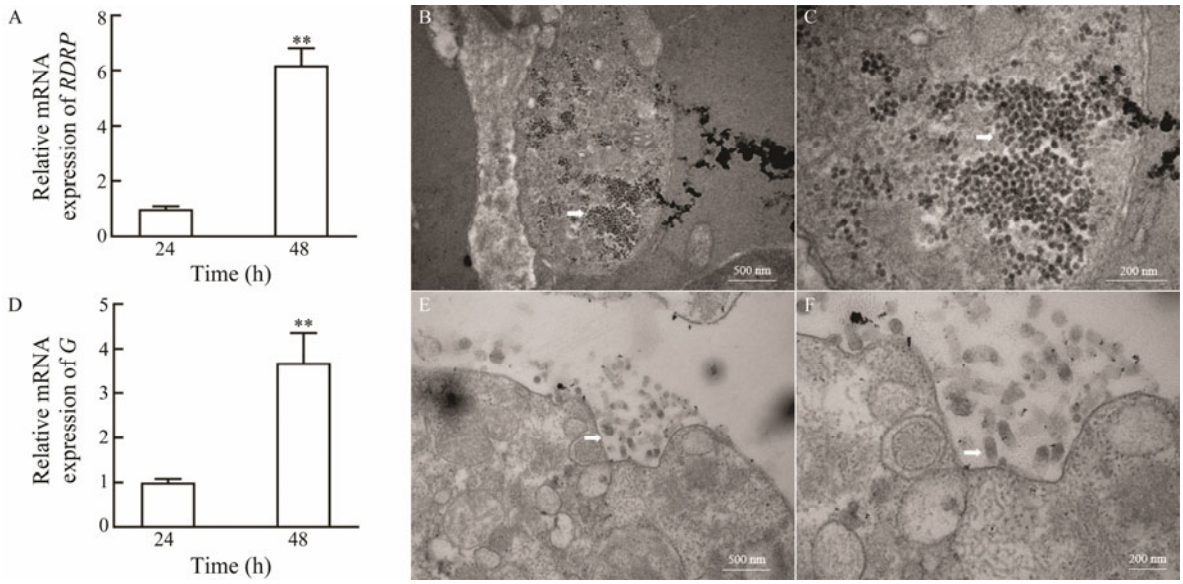

Fig.6 Replication of RGNNV and VHSV in ASF cells. Expression analysis of the RGNNV *RDRP* gene (A) and the VHSV *G* (D) gene in ASF cells at 24 h and 48 h after RGNNV infection (***P* < 0.01) were showed. B–C, Virus particles in RGNNV-infected ASF cells under low magnification (×40000) (B) and high magnification (×100000) (C). E–F, Virus particles in VHSV-infected ASF cells under low magnification (×40000) (E) and high magnification (×80000) (F).

3.6 Expression of Antiviral Genes in ASF Cells After RGNNV and VHSV Infections

The inductions of *IFN-1* and *Mx-1* by RGNNV or VHSV infection were analyzed to investigate the antiviral response of ASF cells upon viral infection. As shown in Figs.7A–7B, RGNNV infection suppressed the expression of *IFN-1* and *Mx-1* at 24 and 48 hpi. By contrast, VHSV significantly induced the expression of *IFN-1* and *Mx-1* at 24 and 48 hpi (Figs.7C–7D). These data indicate that ASF cells can be utilized as a potential *in vitro* tool to explore the cellular antiviral response of Atlantic salmon to fish pathogens.

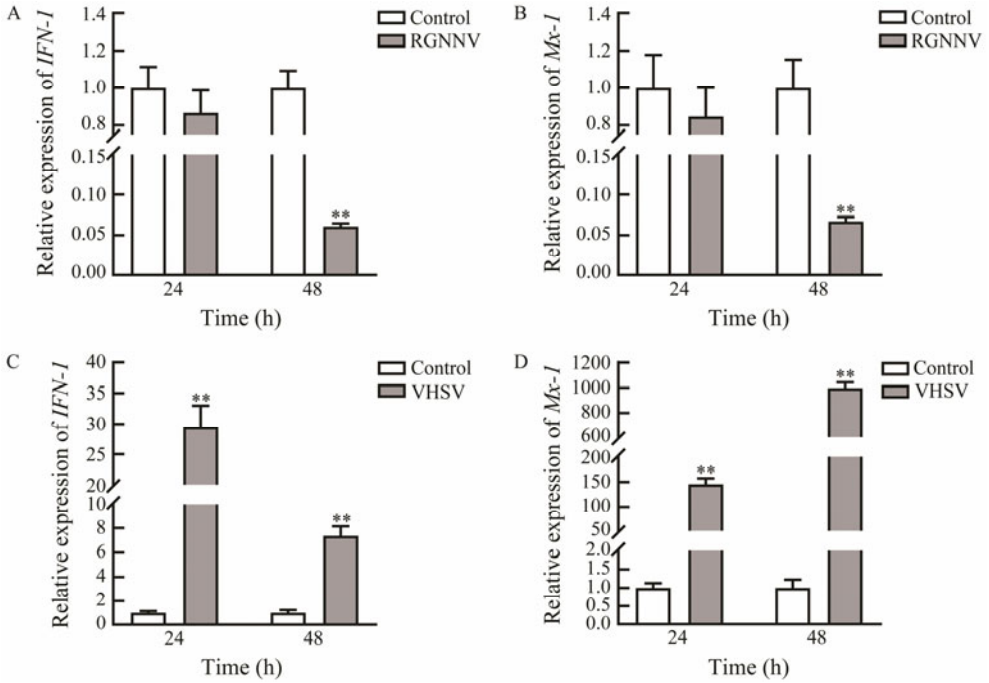

Fig.7 Transcription levels of *IFN-1* and *Mx-1* genes in ASF cells after RGNNV at (A) 24 h and 48 h (B), and VHSV infection at 24 h (C) and 48 h (D). *EF-1α* was used as an internal control. Results were expressed as mean ± SD from three independent experiments performed in triplicates. Asterisks indicate significant differences between groups (***P* < 0.01).

4 Discussion

Fin tissues are the major entry points in fish of several viruses, such as infectious hematopoietic necrosis virus, VHSV, and infectious salmon anemia virus (Harmache *et al*., 2006; Quillet *et al*., 2007; Aamelfot *et al*., 2015). Although several cell lines from various tissues of Atlantic salmon

have been established, few studies have been conducted on fin-derived cell lines in this species. In the present study, a cell line designated as ASF was successfully established and characterized from the caudal fin of Atlantic salmon. Previous studies showed that several cell lines from Atlantic salmon were usually cultured in L-15 medium (Martin *et al*., 2007; Rodriguez Saint-Jean *et al*., 2014; Pham *et al*., 2017b; Gjessing *et al*., 2018). Our study found that DMEM was also suitable for ASF cell culture. The ASF cell line showed stable growth in DMEM with 20% FBS and had been cultured for more than 50 passages so far. The optimal concentration of FBS for ASF cells was estimated to be 20%, which is consistent with ASHe and BAASf, two other cell lines from Atlantic salmon (Pham *et al*., 2017a, 2017b). Previous studies cultured different cell lines of Atlantic salmon at different temperatures. For instance, SSP-9, ASG-10, and ASG-13 were incubated at 20℃, whereas ASHe grew well at 26℃ (Rodriguez Saint-Jean *et al*., 2014; Pham *et al*., 2017b; Gjessing *et al*., 2018). In the study, ASF cells could grow in the temperature range of 10℃–28℃ and exhibited optimal growth at 20℃. Atlantic salmon is a cold-water fish with comfortable temperatures for growth ranging from 15℃ to 20.5℃ and a critical thermal threshold of 27.8℃, which covers the optimum temperature of the cultured cells *in vitro*, suggesting the consistency of cell growth environment *in vivo* and *in vitro* (Beaupré *et al*., 2020).

The typical chromosomal number of ASF cells (2n=58) was identical to that of Atlantic salmon but did not coincide with other cell lines isolated from Atlantic salmon, such as SSP-9 (2n=48) and AS (2n=52) (Sánchez *et al*., 1993; Pendás *et al*., 1994; Rodriguez Saint-Jean *et al*., 2014). The chromosome number in fish cell lines undergoing a degree of cell transformation does not always coincide with that of the intact host species (Schneider, 1973). These results indicate that ASF cells maintain their ploidy nature. Epithelial cells exhibit proliferative and migratory abilities, and the epithelial nature of the ASF cells was validated by successfully closing the scratch within 7 days (Gjessing *et al*., 2018).

Fish cell lines are commonly used in virology research (Jyotsna *et al*., 2019). In the present study, the susceptibility of ASF cells to RGNNV and VHSV was determined. Significant CPEs and amplification of the RGNNV *RDRP* gene demonstrated that ASF cells were susceptible to RGNNV. Furthermore, the result of qRT-PCR and electron microscopy assays confirmed the proliferation of RGNNV in ASF cells. NNV is a potentially dangerous pathogen of Atlantic salmon (Korsnes *et al*., 2005). However, no cell line from Atlantic salmon is sensitive to NNV. Our results indicated that the ASF cell line could be used for NNV isolation and identification. Similar to ASG-10 and ASG-13 cell lines derived from the gill tissue of Atlantic salmon (Gjessing *et al*., 2018), ASF cells showed high susceptibility to VHSV. The fin is a route of entry for VHSV (Montero *et al*., 2011). Thus, ASF cells can be a useful *in vitro* tool for studying VHSV infection in a tissue-specific manner.

Fish cell lines are important tools for studying antiviral mechanisms in fish cells. However, not all cultured fish cells *in vitro* are sensitive to immunostimulation (Rodriguez Saint-Jean *et al*., 2014; Pham *et al*., 2017a, 2020). IFN plays a key role in innate immune response induced by virus infection. Virus invasion induces IFN expression, and then activates the expression of downstream IFN-stimulated genes (ISGs), such as Mx-1, Viperin and ISG15, to resist the virus (Lu *et al*., 2021). In the present study, we detected the transcript levels of *IFN-1* and *Mx-1* to determine whether ASF cells could be used to elucidate the mechanism of host-virus interaction (Zeng *et al*., 2016; Corrales *et al*., 2017). Our results showed that VHSV and RGNNV could significantly alter host cellular *IFN-1* and *Mx* transcriptions in ASF cells, indicating that the ASF cell line might be a useful tool for studying the mechanism of host-virus interaction. Upon VHSV infection, the expression levels of *IFN-1* and *Mx-1* were significantly upregulated, which were in accordance with those in Atlantic salmon *in vivo* and in BASSf cells derived from the bulbus arteriosus of Atlantic salmon (Lovy *et al*., 2013; Pham *et al*., 2017a). By contrast, RGNNV infection significantly suppresses the expression of *IFN-1* and its downstream factor *Mx-1*, which play crucial roles in repressing NNV replication (Wu *et al*., 2016, 2010). Previous study also showed that antiviral genes might exhibit opposite expression patterns when challenged with different viruses. For instance, TNF-a2 and IL-12 p40-c were upregulated post infectious pancreatic necrosis virus and infectious salmon anaemia virus infection, but downregulated during salmonid alphavirus infection in the salmonid cell line TO (Nerbovik *et al*., 2017). As the first line of defense against virus infection, the type-I IFN response was activated to elicit antiviral responses by inducing the expression of ISGs. Meanwhile, viruses have evolved various strategies to suppress the activation of host type-I IFN responses and thus survive in host cells (Chan and Gack, 2016; Lei *et al*., 2020). We speculated that RGNNV escapes the innate immune defense by inhibiting the IFN response in ASF cells. Further studies are needed to clarify the mechanism by which NNV evades the cellular antiviral responses in ASF cells.

5 Conclusions

A cell line derived from the caudal fin of Atlantic salmon, designated as ASF, was established and characterized. ASF cells are susceptible to VHSV and RGNNV, and thus belong to VHSV- and RGNNV-supportive invitromes. VHSV and RGNNV infections induced different cellular responses in ASF cells. Taken together, our results indicate that the ASF cell line can serve as a useful *in vitro* tool for studying the pathogenesis of fish viruses and elucidating the mechanism of host-virus interaction.

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References

Aamelfot, M., McBeath, A., Christiansen, D. H., Matejusova, I., and Falk, K., 2015. Infectious salmon anaemia virus (ISAV) mucosal infection in Atlantic salmon. *Veterinary Research*, **46**: 120, DOI: 10.1186/s13567-015-0265-1.

Beaupré, J., Boudreault, J., Bergeron, N. E., and St-Hilaire, A., 2020. Inclusion of water temperature in a fuzzy logic Atlantic salmon (*Salmo salar*) parr habitat model. *Journal of Thermal Biology*, **87**: 102471, DOI: 10.1016/j.jtherbio.2019.102471.

Bols, N. C., Pham, P. H., Dayeh, V. R., and Lee, L. E. J., 2017. Invitromatics, invitrome, and invitroomics: Introduction of three new terms for *in vitro* biology and illustration of their use with the cell lines from rainbow trout. *In Vitro Cellular & Developmental Biology−Animal*, **53**: 383-405, DOI: 10.1007/s11626-017-0142-5.

Brudeseth, B. E., Wiulsrød, R., Fredriksen, B. N., Lindmo, K., Løkling, K. E., Bordevik, M., *et al*., 2013. Status and future perspectives of vaccines for industrialised fin-fish farming. *Fish & Shellfish Immunology*, **35** (6): 1759-1768, DOI: 10.1016/j.fsi.2013.05.029.

Chan, Y. K., and Gack, M. U., 2016. Viral evasion of intracellular DNA and RNA sensing. *Nature Reviews Microbiology*, **14** (6): 360-373, DOI: 10.1038/nrmicro.2016.45.

Collet, B., Collins, C., and Lester, K., 2018. Engineered cell lines for fish health research. *Developmental and Comparative Immunology*, **80**: 34-40, DOI: 10.1016/j.dci.2017.01.013.

Corrales, L., Matson, V., Flood, B., Spranger, S., and Gajewski, T. F., 2017. Innate immune signaling and regulation in cancer immunotherapy. *Cell Research*, **27** (1): 96-108, DOI: 10.1038/cr.2016.149.

Dharmaratnam, A., Kumar, R., Valaparambil, B. S., Sood, N., Pradhan, P. K., Das, S., *et al*., 2020. Establishment and characterization of fantail goldfish fin (FtGF) cell line from goldfish, *Carassius auratus* for *in vitro* propagation of Cyprinid herpes virus-2 (CyHV-2). *PeerJ*, **8**: e9373, DOI: 10.7717/peerj.9373.

Emmenegger, E. J., Moon, C. H., Hershberger, P. K., and Kurath, G., 2013. Virulence of viral hemorrhagic septicemia virus (VHSV) genotypes Ia, IVa, IVb, and IVc in five fish species. *Diseases of Aquatic Organisms*, **107** (2): 99-111, DOI: 10.3354/dao02671.

Eriksson-Kallio, A. M., Holopainen, R., Koski, P., Nousiainen, A., Koskinen, H., Kause, A., *et al*., 2020. Susceptibility of rainbow trout to three different genogroups of infectious pancreatic necrosis virus. *Diseases of Aquatic Organisms*, **141**: 103-116, DOI: 10.3354/dao03512.

Gjessing, M. C., Aamelfot, M., Batts, W. N., Benestad, S. L., Dale, O. B., Thoen, E., *et al*., 2018. Development and characterization of two cell lines from gills of Atlantic salmon. *PLoS One*, **13** (2): e0191792, DOI: 10.1371/journal.pone.0191792.

Gjessing, M. C., Krasnov, A., Timmerhaus, G., Brun, S., Afanasyev, S., Dale, O. B., *et al*., 2020. The Atlantic salmon gill transcriptome response in a natural outbreak of salmon gill pox virus infection reveals new biomarkers of gill pathology and suppression of mucosal defense. *Frontiers in Immunology*, **11**: 2154, DOI: 10.3389/fimmu.2020.02154.

Harmache, A., LeBerre, M., Droineau, S., Giovannini, M., and Bremont, M., 2006. Bioluminescence imaging of live infected salmonids reveals that the fin bases are the major portal of entry for Novirhabdovirus. *Journal of Virology*, **80** (7): 3655-3659, DOI: 10.1128/JVI.80.7.3655-3659.2006.

Ito, T., Kurita, J., Mori, K., and Olesen, N. J., 2016. Virulence of viral haemorrhagic septicaemia virus (VHSV) genotype III in rainbow trout. *Veterinary Research*, **47**: 4, DOI: 10.1186/s13567-015-0303-z.

Jenberie, S., Peñaranda, M. M. D., Thim, H. L., Styrvold, M. B., Strandskog, G., Jørgensen, J. B., *et al*., 2020. Salmonid alphavirus subtype 3 induces prolonged local B cell responses in Atlantic salmon (*Salmo salar*) after intraperitoneal infection. *Frontiers in Immunology*, **11**: 1682, DOI: 10.3389/fimmu.2020.01682.

Jia, P., Chen, X., Fu, J., Yi, M., Chen, W., and Jia, K., 2020. Near-Complete genome sequence of a fish nervous necrosis virus isolated from hybrid grouper in China. *Microbiology Resource Announcements*, **9** (15): e01453-19, DOI: 10.1128/mra.01453-19.

Jin, Y., Datsomor, A. K., Olsen, R. E., Vik, J. O., Torgersen, J. S., Edvardsen, R. B., *et al*., 2020. Targeted mutagenesis of Δ5 and Δ6 fatty acyl desaturases induce dysregulation of lipid metabolism in Atlantic salmon (*Salmo salar*). *BMC Genomics*, **21** (1): 805, DOI: 10.1186/s12864-020-07218-1.

Jyotsna, Vijayakumar, P., Ravi, M., Sudhakaran, R., and Rajaswaminathan, T. J. A., 2019. Development and characterization of a skin cell line (SGA) from the mosquitofish *Gambusia affinis* and its susceptibility to fish Betanodavirus. *Aquaculture*, **520**: 734778, DOI: 10.1016/j.aquaculture.2019.734778.

Kim, K. I., Lee, U. H., Cho, M., Jung, S. H., Min, E. Y., and Park, J. W., 2020. Transcriptome analysis based on RNA-seq of common innate immune responses of flounder cells to IHNV, VHSV, and HIRRV. *PLoS One*, **15** (9): e0239925, DOI: 10.1371/journal.pone.0239925.

Korsnes, K., Devold, M., Nerland, A. H., and Nylund, A., 2005. Viral encephalopathy and retinopathy (VER) in Atlantic salmon *Salmo salar* after intraperitoneal challenge with a nodavirus from Atlantic halibut *Hippoglossus hippoglossus*. *Diseases of Aquatic Organisms*, **68** (1): 7-15, DOI: 10.3354/dao068007.

Kwon, W. J., Choi, J. C., Hong, S., Kim, Y. C., Jeong, M. G., Min, J. G., *et al*., 2020. Development of a high-dose vaccine formulation for prevention of megalocytivirus infection in rock bream (*Oplegnathus fasciatus*). *Vaccine*, **38** (51): 8107-8115, DOI: 10.1016/j.vaccine.2020.11.001.

Le, Y., Li, Y., Jin, Y., Jia, P., Jia, K., and Yi, M., 2017. Establishment and characterization of a brain cell line from sea perch, *Lateolabrax japonicus*. *In Vitro Cellular & Developmental Biology−Animal*, **53** (9): 834-840, DOI: 10.1007/s11626-017-0185-7.

Lei, V., Petty, A. J., Atwater, A. R., Wolfe, S. A., and MacLeod, A. S., 2020. Skin viral infections: Host antiviral innate immunity and viral immune evasion. *Frontiers in Immunology*, **11**: 593901, DOI: 10.3389/fimmu.2020.593901.

Li, J., Jia, P., Chen, X., Lai, M., Jin, F., Liu, W., *et al.*, 2019. Establishment and characterization of a fin tissue cell line derived from silver pomfret, *Pampus argenteus*. *Journal of Fish Diseases*, **42** (10): 1391-1399, DOI: 10.1111/jfd.13059.

Lovy, J., Piesik, P., Hershberger, P. K., and Garver, K. A., 2013. Experimental infection studies demonstrating Atlantic salmon as a host and reservoir of viral hemorrhagic septicemia virus type IVa with insights into pathology and host immunity. *Veterinary Microbiology*, **166** (1-2): 91-101, DOI: 10.1016/j.vetmic.2013.05.019.

Lozano-Muñoz, I., Muñoz, S., Díaz, N. F., Medina, A., Bazaes, J., and Riquelme, C., 2020. Nutritional enhancement of farm-

ed salmon meat via non-GMO *Nannochloropsis gaditana*: Eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3) and vitamin D3 for human health. *Molecules*, **25** (20): 4615, DOI: 10.3390/molecules 25204615.

Lu, X., Zeng, J., Jia, K., and Yi, M., 2021. Antiviral activities of sea perch type I and type II IFNs against RGNNV and their different roles in antigen presentation. *Aquaculture*, **534**: 736314, DOI: 10.1016/j.aquaculture.2020.736314.

Martin, S. A., Taggart, J. B., Seear, P., Bron, J. E., Talbot, R., Teale, A. J., *et al*., 2007. Interferon type I and type II responses in an Atlantic salmon (*Salmo salar*) SHK-1 cell line by the salmon TRAITS/SGP microarray. *Physiological Genomics*, **32** (1): 33-44, DOI: 10.1152/physiolgenomics.00064.2007.

Montero, J., Garcia, J., Ordas, M. C., Casanova, I., Gonzalez, A., Villena, A., *et al*., 2011. Specific regulation of the chemokine response to viral hemorrhagic septicemia virus at the entry site. *Journal of Virology*, **85** (9): 4046-4056, DOI: 10.1128/jvi.02519-10.

Munro, E. S., McIntosh, R. E., Weir, S. J., Noguera, P. A., Sandilands, J. M., Matejusova, I., *et al*., 2015. A mortality event in wrasse species (Labridae) associated with the presence of viral haemorrhagic septicaemia virus. *Journal of Fish Diseases*, **38** (4): 335-341, DOI: 10.1111/jfd.12237.

Nerbovik, I. G., Solheim, M. A., Eggestol, H. O., Ronneseth, A., Jakobsen, R. A., Wergeland, H. I., *et al*., 2017. Molecular cloning of MDA5, phylogenetic analysis of RIG-I-like receptors (RLRs) and differential gene expression of RLRs, interferons and proinflammatory cytokines after *in vitro* challenge with IPNV, ISAV and SAV in the salmonid cell line TO. *Journal of Fish Diseases*, **40** (11): 1529-1544, DOI: 10.1111/jfd.12622.

Pendás, A. M., Morán, P., and García-Vázquez, E., 1994. Organization and chromosomal location of the major histone cluster in brown trout, Atlantic salmon and rainbow trout. *Chromosoma*, **103** (2): 147-152, DOI: 10.1007/bf00352324.

Pham, P. H., Misk, E., Papazotos, F., Jones, G., Polinski, M. P., Contador, E., *et al*., 2020. Screening of fish cell lines for piscine orthoreovirus-1 (PRV-1) amplification: Identification of the non-supportive PRV-1 invitrome. *Pathogens*, **9** (10): 833, DOI: 10.3390/pathogens9100833.

Pham, P. H., Tong, W. W. L., Misk, E., Jones, G., Lumsden, J. S., and Bols, N. C., 2017a. Atlantic salmon endothelial cells from the heart were more susceptible than fibroblasts from the bulbus arteriosus to four RNA viruses but protected from two viruses by dsRNA pretreatment. *Fish & Shellfish Immunology*, **70**: 214-227, DOI: 10.1016/j.fsi.2017.09.001.

Pham, P. H., Vo, N. T., Tan, E. J., Russell, S., Jones, G., Lumsden, J. S., *et al*., 2017b. Development of an Atlantic salmon heart endothelial cell line (ASHe) that responds to lysophosphatidic acid (LPA). *In Vitro Cellular & Developmental Biology Animal*, **53** (1): 20-32, DOI: 10.1007/s11626-016-0077-2.

Quillet, E., Dorson, M., Aubard, G., and Torhy, C., 2007. *In vitro* assay to select rainbow trout with variable resistance/susceptibility to viral haemorrhagic septicaemia virus. *Diseases of Aquatic Organisms*, **76** (1): 7-16, DOI: 10.3354/dao076007.

Robin, T., Capes-Davis, A., and Bairoch, A., 2020. CLASTR: The Cellosaurus STR similarity search tool−A precious help for cell line authentication. *International Journal of Cancer*, **146** (5): 1299-1306, DOI: 10.1002/ijc.32639.

Rodriguez Saint-Jean, S., González, C., Monrás, M., Romero, A., Ballesteros, N., Enríquez, R., *et al*., 2014. Establishment and characterization of a new cell line (SSP-9) derived from Atlantic salmon *Salmo salar* that expresses type I ifn. *Journal of Fish Biology*, **85** (5): 1526-1545, DOI: 10.1111/jfb.12503.

Samsing, F., Rigby, M., Tengesdal, H. K., Taylor, R. S., Farias, D., Morrison, R. N., *et al*., 2021. Seawater transmission and infection dynamics of pilchard orthomyxovirus (POMV) in Atlantic salmon (*Salmo salar*). *Journal of Fish Diseases*, **44** (1): 73-88, DOI: 10.1111/jfd.13269.

Sánchez, L., Abuín, M., and Amaro, R., 1993. Cytogenetic characterization of the AS cell line derived from the Atlantic salmon (*Salmo salar* L.). *Cytogenetics and Cell Genetics*, **64** (1): 35-38, DOI: 10.1159/000133556.

Schneider, I. J. T. C., 1973. *Karyology of Cells in Culture*. Academic Press, New York, 788-790, DOI: 10.1016/B978-0-12-427150-0.50172-8.

Wu, Y. C., Lu, Y. F., and Chi, S. C., 2010. Anti-viral mechanism of barramundi Mx against betanodavirus involves the inhibition of viral RNA synthesis through the interference of RdRp. *Fish & Shellfish Immunology*, **28** (3): 467-475, DOI: 10.1016/j.fsi.2009.12.008.

Wu, Y. C., Tsai, P. Y., Chan, J. C., and Chi, S. C., 2016. Endogenous grouper and barramundi Mx proteins facilitated the clearance of betanodavirus RNA-dependent RNA polymerase. *Developmental and Comparative Immunology*, **59**: 110-120, DOI: 10.1016/j.dci.2016.01.012.

Zeng, M., Chen, S., Wang, M., and Chen, A., 2016. Advances in avian antiviral innate immune effectors. *Chinese Journal of Virology*, **32** (5): 627-633.

Zhang, W., Jia, K., Jia, P., Xiang, Y., Lu, X., Liu, W., *et al.*, 2020. Marine medaka heat shock protein 90ab1 is a receptor for red-spotted grouper nervous necrosis virus and promotes virus internalization through clathrin-mediated endocytosis. *PLoS Pathogens*, **16** (7): e1008668, DOI: 10.1371/journal.ppat.1008668.

Zhang, W., Jia, P., Liu, W., Li, Y., Yi, M., and Jia, K., 2018. Functional characterization of tumor necrosis factor receptor-associated factor 3 of sea perch (*Lateolabrax japonicas*) in innate immune. *Fish & Shellfish Immunology*, **75**: 1-7, DOI: 10.1016/j.fsi.2018.01.039.

Zhang, W., Li, Z., Xiang, Y., Jia, P., Liu, W., Yi, M., *et al*., 2019. Isolation and identification of a viral haemorrhagic septicaemia virus (VHSV) isolate from wild largemouth bass *Micropterus salmoides* in China. *Journal of Fish Diseases*, **42** (11): 1563-1572, DOI: 10.1111/jfd.13078.

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