MOL231

Antiviral responses to ISAV in salmonid cells

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Background and aim:

Infectious Salmon Anemia Virus (ISAV) is an enveloped RNA virus and a major viral pathogen in Atlantic salmon aquaculture. ISAV occurs in both non-virulent (HPR0) and virulent (HPRΔ) forms, where the transition to virulence is associated with genetic changes, particularly in surface glycoproteins such as hemagglutinin-esterase (HE), which affect viral infectivity and dissemination within the host[1] (Fig. 1).

Aim: Investigate antiviral responses and viral infection in salmonid cells following viral exposure using salmon leukocytes and CHSE cells.

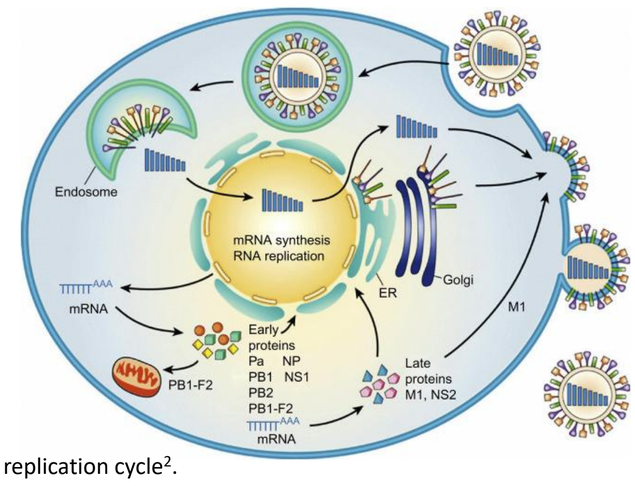
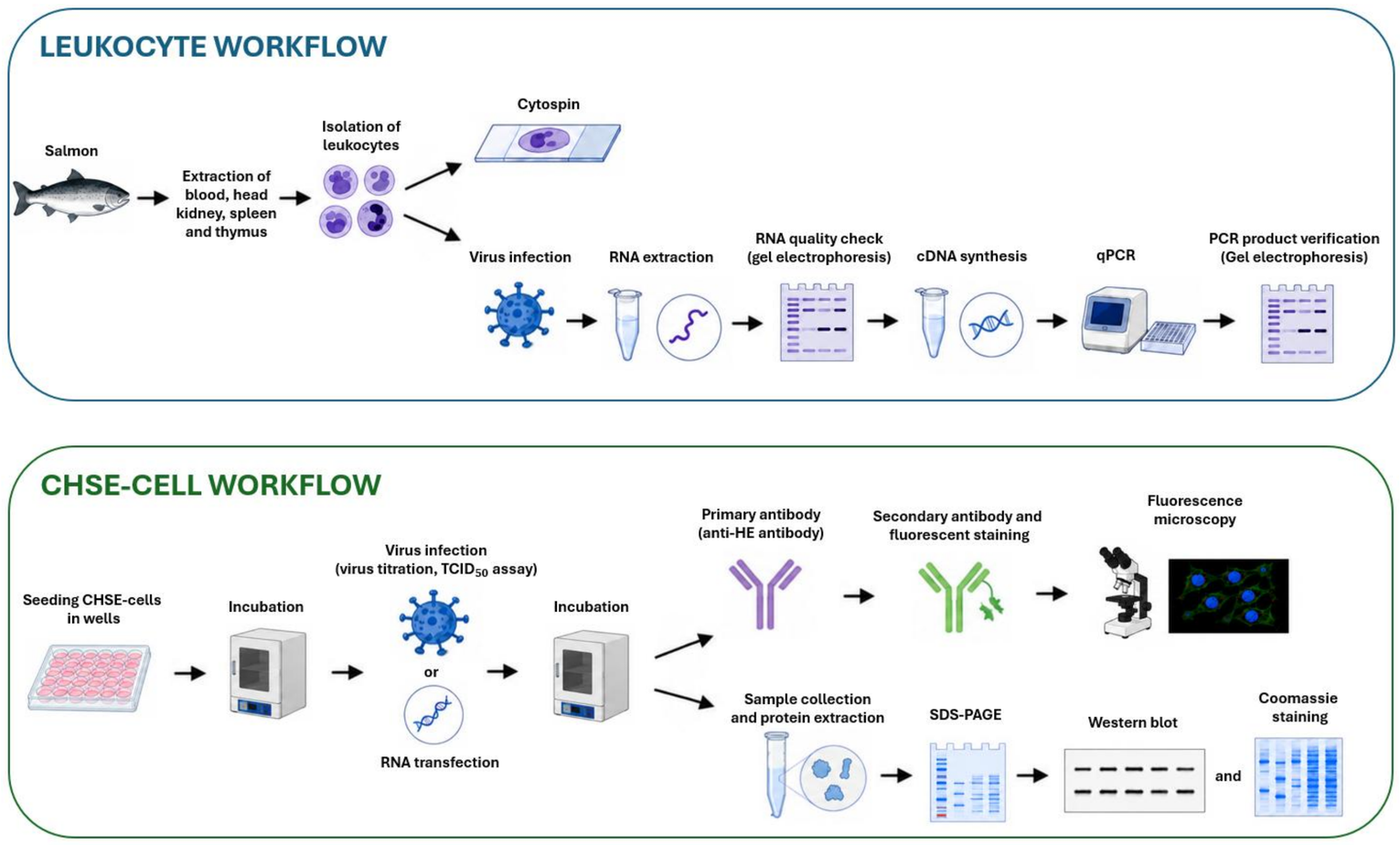


Figure 1: ISAV replication cycle[2].

Methods:



Results and discussion:

Leukocytes:

Increased expression of IFNa and Mx demonstrated activation of an antiviral response following ISAV exposure (Fig. 4), indicating that salmon leukocytes detected and responded to the virus. However, these results should be verified using additional parallels.

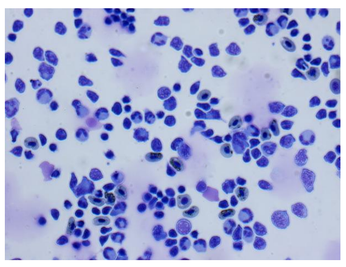


Figure 2: Cytospin preparation of perifere blood leukocytes (PBL) isolated from Atlantic salmon.

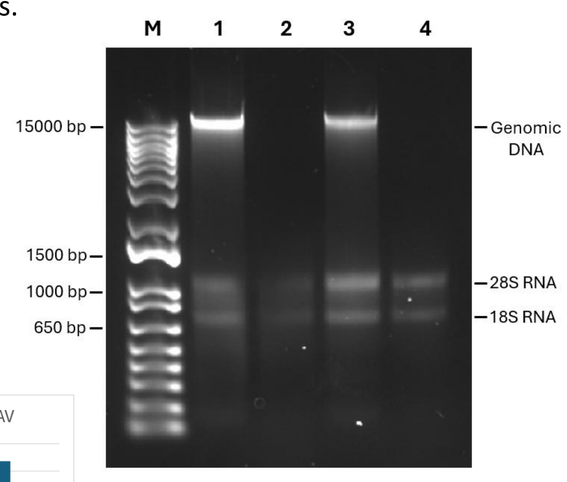


Figure 3: Agarose gel electrophoresis of RNA isolated from PBL before (lanes 1 and 3) and after DNase treatment (lanes 2 and 4). Lane M: 1 kb+ DNA ladder; lanes 1-2: control sample RNA; lanes 3-4: ISAV-exposed sample RNA. The RNA samples were of sufficient quality for downstream qPCR analysis.

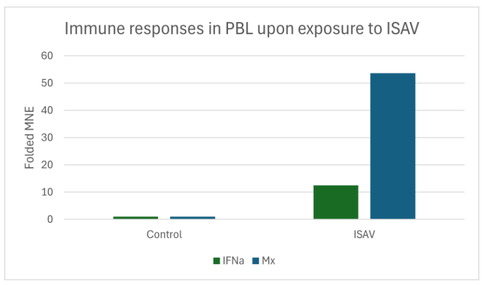


Figure 4: Relative expression of IFNa and Mx in PBL following ISAV exposure, measured by qPCR.

CHSE-cells:

ISAV infection induced cytopathic effects (CPE) in CHSE cells (Fig. 5). The antibody was able to detect the viral HE protein (Figs. 6-7); however, immunofluorescence staining indicated limited suitability for detection of ISAV infection in CHSE cells (Fig. 6).

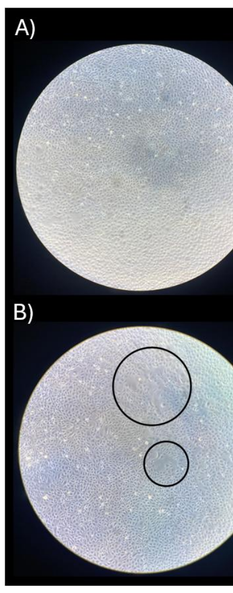


Figure 5: CHSE cells cultured in wells. Well A: non-infected control; well B: ISAV-exposed cells (10^{-1} dilution). Marked areas indicate cytopathic effects (CPE).

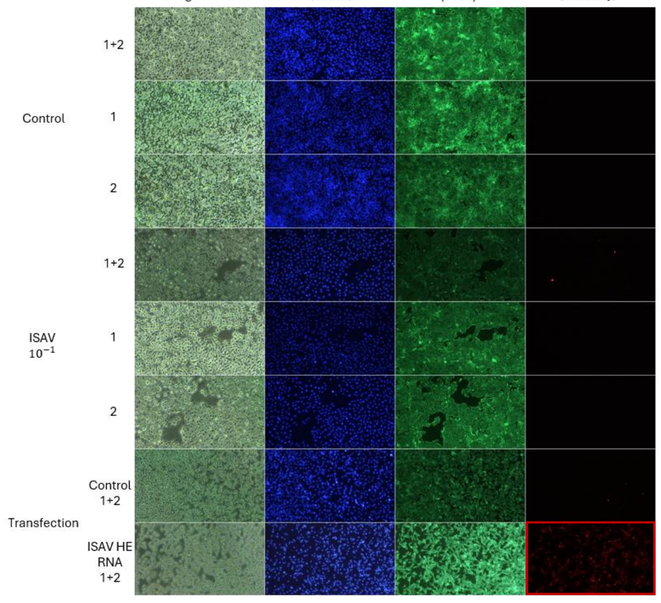


Figure 6:Immunofluorescence staining of CHSE cells following either ISAV infection or transfection with ISAV HE RNA. 1: primary antibody; 2: secondary antibody; 1+2: both antibodies. Red box highlights HE-positive fluorescence.

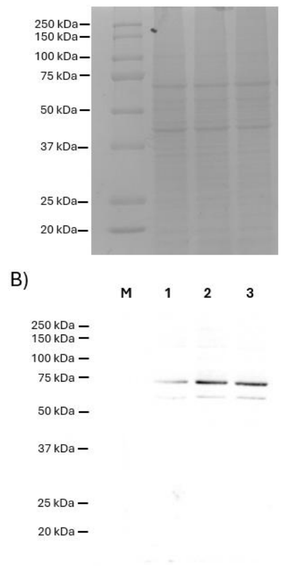


Figure 7: Protein analysis of CHSE cells exposed to ISAV. Lane M: molecular weight marker (10-250 kDa); lane 1: control; lane 2: ISAV 10^{-2}; lane 3: ISAV 10^{-3}. (A) Coomassie-stained SDS-PAGE gel. (B) Western blot analysis.

ISAV HE protein sequence:

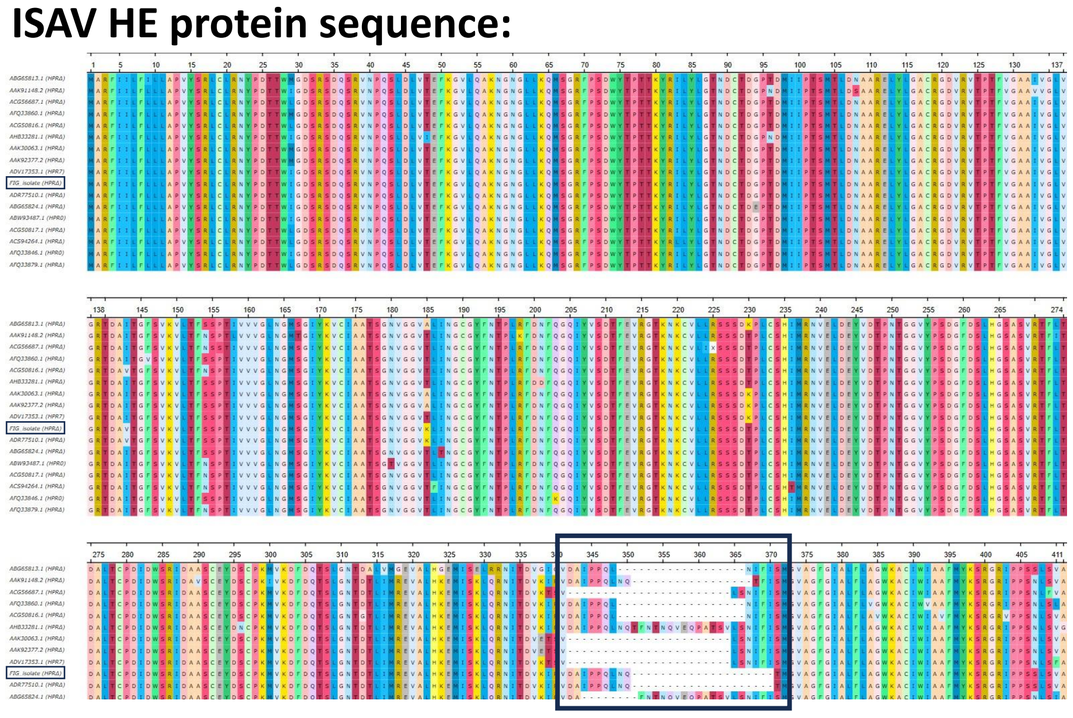


Figure 8: Multiple sequence alignment of the HE protein from several ISAV isolates, including isolate names/accession numbers and HPR types. The isolate used in this study[3] is indicated by small boxes, and the large boxed area marks the highly polymorphic region (HPR).

The multiple sequence alignment revealed genetic differences in the highly polymorphic region (HPR) between HPRΔ and HPR0 ISAV isolates, where the HPRΔ variants contained shortened regions (Fig. 8). Phylogenetic analysis indicated clustering of isolates that was largely associated with geographic origin (Fig. 9).

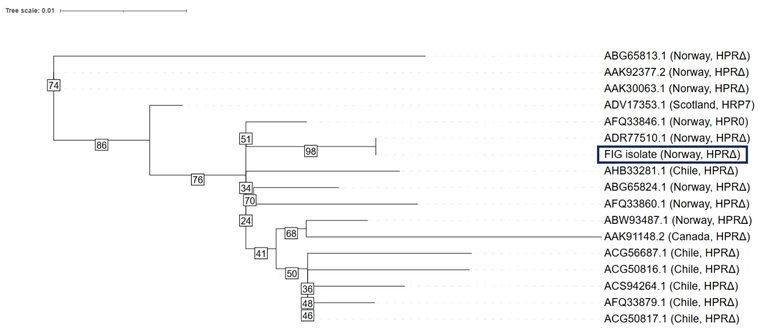


Figure 9: Phylogenetic tree based on multiple sequence alignment of the HE protein from several ISAV isolates, including isolate names/accession numbers, geographic origin and HPR types. The isolate used in this study[3] is indicated by boxes. Numbers at the nodes represent bootstrap support values.

Conclusion:

In salmon leukocytes, ISAV exposure induced activation of immune-related gene expression. In CHSE cells, ISAV infection induced cytopathic effects, while transfection and infection experiments confirmed detection of the viral HE protein. Sequence analysis of the ISAV HE protein from several isolates further confirmed genetic variation in the highly polymorphic region (HPR), distinguishing HPRΔ from HPR0 variants and revealing clustering linked to geographic origin. Further studies of ISAV are important for understanding how salmon cells respond to viral infection and may contribute to future research on antiviral immunity and vaccine development in Atlantic salmon.

References:

1: Rimstad, E., & Markussen, T. (2019). Infectious salmon anaemia virus - molecular biology and pathogenesis of the infection. Journal of Applied Microbiology, ss. 85-97.
2: Elsevier. (u.d.). Isavirus. Retrieved from ScienceDirect: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/isavirus
3: Nerbøvik et al. 2017. Journal of fish diseases. doi:10.1111/jfd.12622

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