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Transcriptome analysis based on RNA-seq of common innate immune responses of flounder cells to IHNV, VHSV, and HIRRV

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Abstract

Viral hemorrhagic septicemia virus (VHSV) and hirame rhabdovirus (HIRRV) belong to the genus *Novirhabdovirus* and are the causative agents of a serious disease in cultured flounder. However, infectious hematopoietic necrosis virus (IHNV), a prototype of the genus Novirhabdovirus, does not cause disease in flounder. To determine whether IHNV growth is restricted in flounder cells, we compared the growth of IHNV with that of VHSV and HIRRV in hirame natural embryo (HINAE) cells infected with novirhabdoviruses at 1 multiplicity of infection. Unexpectedly, we found that IHNV grew as well as VHSV and HIRRV. For successful growth in host cells, viruses modulate innate immune responses exerted by virus-infected cells. Our results suggest that IHNV, like VHSV and HIRRV, has evolved the ability to overcome the innate immune response of flounder cells. To determine the innate immune response genes of virus-infected HINAE cells which are commonly modulated by the three novirhabdoviruses, we infected HINAE cells with novirhabdoviruses at multiplicity of infection (MOI) 1 and performed an RNA sequencing-based transcriptome analysis at 24 h post-infection. We discovered ~12,500 unigenes altered by novirhabdovirus infection and found that many of these were involved in multiple cellular pathways. After novirhabdovirus infection, 170 genes involved in the innate immune response were differentially expressed compared to uninfected cells. Among them, 9 genes changed expression by more than 2-fold and were commonly modulated by all three novirhabdoviruses. Interferon regulatory factor 8 (*IRF8*), C-X-C motif chemokine receptor 1 (*CXCR1*), Toll/interleukin-1 receptor domain-containing adapter protein (*TIRAP*), cholesterol 25-hydroxylase (*CH25H*), C-X-C motif chemokine ligand 11, duplicate 5 (*CXCL11.5*), and Toll-like receptor 2 (*TLR2*) were up-regulated, whereas C-C motif chemokine receptor 6a (*CCR6a*), interleukin-12a (*IL12a*), and Toll-like receptor 1 (*TLR1*) were down-regulated. These genes have been reported to be involved in antiviral responses and, thus, their modulation may be critical for the growth of novirhabdovirus in flounder cells. This is the first report to identify innate immune response genes in flounder that are commonly modulated by IHNV, VHSV, and HIRRV. These data will provide new insights into how novirhabdoviruses survive the innate immune response of flounder cells.

Introduction

Novirhabdovirus belongs to the family *Rhabdoviridae* and causes disease in cultured fish, resulting in significant economic losses to aquaculture industries. The genomic RNA of novirhabdoviruses has a size and structure common to the rhabdoviruses. However, unlike other rhabdoviruses, novirhabdovirus has an additional gene encoding non-structural protein (NV) located between the *G* and *L* genes and the novirhabdovirus genome encodes six viral proteins including nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), non-structural (NV) and RNA polymerase protein (L) in the order 3′-N-P-M-G-NV-L-5′ [1–3]. The non-structural protein NV has been reported to play a role in viral replication and in blocking the host innate immune response [4, 5].

The prototype of the genus *Novirhabdovirus* is an infectious hematopoietic necrosis virus (IHNV). In addition to IHNV, viral hemorrhagic septicemia virus (VHSV) and hirame rhabdovirus (HIRRV) are members of the genus *Novirhabdovirus*. The natural host ranges of individual novirhabdoviruses vary in breadth: while IHNV has a very narrow host range, VHSV is able to infect a wide range of species [6]. IHNV was first isolated from sockeye salmon (*Oncorhynchus nerka*) in the United States in the 1950s [7]. Even though IHNV is an acute pathogen of wild and cultured fishes in North America, Europe, and Asia [8–13], the disease caused by IHNV infection is limited to salmonid fish. On the contrary, VHSV, first isolated from rainbow trout in Europe [14], has spread to approximately 80 different fish species worldwide [8, 15, 16]. In Asia, VHSV infection was first reported in olive flounder (*Paralichthys olivaceus*) cultured in Japan in 1996 [17] and then caused disease and serious economic problems in olive flounder farming in Japan [18, 19] and Korea [20, 21]. HIRRV was first described in cultured olive flounder in Japan in the early 1980s [22], from where it gradually spread to South Korea, China [23, 24], and Europe [25] and infected a wide range of marine fishes including olive flounder, stone flounder (*Kareius bicoloratus*), black seabream (*Acanthopagrus schlegeli*), and spotted sea bass (*Lateolabrax maculatus*) [26].

Generally, the natural host range switches by viruses are reported to be rare events. In order to replicate successfully in a new host, virus must adapt to a new environment and overcome many hurdles: entering the host cell, replicating with the assistance of host factors, evading the host defense system, and spreading to other individuals [27]. However, the high mutation rates and large population sizes of RNA viruses facilitate gains in fitness and adaptation to the new environment [28]. Since the novirhabdovirus is an RNA virus and VHSV, a member of the genus novirhabdovirus, has a wide host range, it is possible that IHNV could adapt to a new host such as olive flounder, which is an important fish species in aquaculture.

The initial step of a virus adaptation to a new host is replication within the new host cells. Virus-infected cells recognize viral nucleic acids and induce the synthesis of type I interferon (IFN) (IFN-α and IFN-β) [29]. Binding of type I IFNs to the IFN receptor on the surface of neighboring cells leads to the induction of more than 300 IFN-stimulated genes (ISGs) which can restrict viral replication and, thus, play an important role in the first line of defense against infection [30–32]. The replication of novirhabdoviruses has been reported to be inhibited by the IFN system [33]. Thus, for successful replication within new host cells, virus must develop strategies to evade defense mechanisms exerted by virus-infected cells. Many viruses have developed strategies to inhibit the IFN response [31, 34] and, even though there is controversy, several studies suggest that the virulence of viruses correlates with their IFN resistance or their ability to inhibit IFN induction in infected cells [35–37].

In the present study, we investigated whether IHNV grows as efficiently as VHSV and HIRRV in hirame natural embryo (HINAE) cells. The growth of IHNV in flounder cells were found to be comparable to that of VHSV and HIRRV, indicating that IHNV, like VHSV and

HIRRV, has successfully adapted to flounder cells. We infected HINAE cells with three novirhabdoviruses at 1 multiplicity of infection (MOI) and used RNA sequencing (RNA-seq) to analyze changes in host gene expression profiles after novirhabdovirus infection, with special emphasis on the innate immune response genes that were commonly modulated by all three novirhabdoviruses. We present a list of 170 innate immune response genes in HINAE cells modulated by novirhabdovirus infection. Moreover, we identified 9 innate immune response genes commonly modulated by three novirhabdoviruses with more than 2-fold expression change. The expression of the selected genes was also validated by quantitative real-time polymerase chain reaction (qRT-PCR). These data can be used to understand innate immune responses in novirhabdovirus-infected flounder cells and provide insights into how novirhabdoviruses have adapted to flounder cells.

Materials and methods

Cell lines

Hirame natural embryo (HINAE) cells (RRID:CVCL_R908) from olive flounder (*Paralichthys olivaceus*) [38] were kindly provided by Dr. Ikuo Hirono (Tokyo University of Marine Science and Technology, Japan). Epithelioma papulosum cyprini (EPC) cells (ATCC Cat# CRL-2872, RRID:CVCL_4361) from fathead minnow (*Pimephales promelas*) [39] were purchased from ATCC). They were grown at 20°C in Eagle's minimal essential medium (MEM) (GIBCO-BRL) supplemented with 10% fetal bovine serum (GIBCO-BRL).

Viruses

IHNV (FP-RtCc 0517), HIRRV-8401H (ATCCVR-1391), and VHSV-VHS2015-5 were used in this study. The source, place of location, and year of isolation of the novirhabdoviruses used in this study are provided in Table 1. Viruses were propagated in EPC cells at 20°C and were quantified using plaque-forming units (PFU/mL) by a standard plaque assay [40].

Virus growth curve analysis

Infections were carried out in 25-cm^2 culture flasks (SPL Life Sciences) containing confluent monolayers of HINAE cells at an MOI of 0.1 and 1 PFU per cell. At the end of a 1-hour (hereafter h) absorption period at 20°C, the inoculum was removed, and cells were washed three times with MEM containing no serum. MEM (5 mL) containing 5% fetal bovine serum (GIBCO-BRL) was then added to each culture flask, and the flasks incubated at 20°C. Samples, consisting of 200 μL of medium, were taken at 0, 24, 72, and 120 h post-infection (p.i.) and stored at -80°C. Viral titer was determined for all samples, in duplicate, by a plaque assay using EPC cells. Growth curves were constructed using a mean log titer for each time point.

Table 1. Novirhabdoviruses used in this study.

Virus	Strain	Source	Place of isolation	Year of isolation
IHNV	FP-RtCc 0517	Rainbow trout	Korea	2017
		(*Oncorhynchus mykiss*)		
VHSV	VHS2015-5	Olive flounder	Korea	2015
		(*Paralichthys olivaceus*)		
HIRRV	8401 H	Olive flounder	Japan	1984
	(ATCC VR-1391)	(*Paralichthys olivaceus*)		

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Quantification of gene expression by qRT-PCR

Total RNA was isolated from virus-infected cells using TRIzol Reagent (Invitrogen), and 2 μg of RNA was used to generate cDNA using M-MLV reverse transcriptase (Promega). Real-time qRT-PCR was performed using SYBR Green PCR Master Mix (Qiagen) on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). The primer pairs are listed in Table 2. Following an initial 10 min denaturation/activation step at 94˚C, the mixture was subjected to 40 cycles of amplification (denaturation for 15 s at 94˚C, annealing and extension for 1 min at 60˚C). The specificity of the qRT-PCR reaction for each amplified product was verified by melting curve analysis. In each qRT-PCR run, a "no template control" was included to detect the existence of primer-dimer or contamination. β-actin gene expression is considered to be stable and we used this gene as an endogenous control gene. The relative changes in gene expression between mock and virus-infected cells were determined by $2^{-\Delta\Delta Ct}$ method using β-actin gene as the normalizing reference, where $\Delta\Delta Ct = (Ct_{target}—Ct_{reference})_{test}—(Ct_{target}—Ct_{reference})_{control}$ [41]. Each gene in each sample was normalized by subtracting the mean threshold cycle (Ct) value of the β-actin endogenous reference gene from the mean Ct value of the target gene. The fold change of each gene was obtained by calculating the difference in the normalized Ct value of target gene between mock- and virus-infected cells.

RNA extraction and Illumina RNA-seq library preparation

HINAE cells were infected with 1 MOI of IHNV, VHSV, or HIRRV at 20˚C. Cells were collected from two independent experiments at 0 and 24 h p.i. and total RNA was isolated from virus-infected cells using TRIzol Reagent (Invitrogen). Residual DNA from each sample was removed by using the RNeasy® MinElute® Cleanup Kit (Qiagen). The total RNA in each sample were determined using a NanoDrop™ 2000 system (Thermo Fisher Scientific) and the 28S/18S ratio was estimated using an Agilent 2100 bioanalyzer (Agilent). The cDNA library was prepared with ∼1.0 μg of total RNA using a TrueSeq RNA Library Preparation Kit (Illumina) following the manufacturer's recommendations. cDNAs were amplified according to

Table 2. PCR primers used in this study.

Name	Accession number	Sequences (forward and reverse, 5′ to 3′)
IRF8	XM_020098702.1	CCAACAAGCTCTGGTGACCT, CCTGGATGTTACAGCCTTCGT
CXCR1	XM_020111721.1	GATCAGCACCAGCAAACAGA, CAGGCCATCAGCTATTGTCA
TIRAP	XM_020095061.1	TGAGCTCACACCTGAGATGG, ATTGGCCTCCTCAATGTCAC
CH25H	XM_020111748.1	GACTCTCAGTCTCGGCGTCT, GACAGCCACATGTTGACCAG
CXCL11.5	XM_020104196.1	TGGCTAATCCTGATGGGTTC, ACAGACTCCCGGTGTCACTC
TLR2	XM_020112731.1	TCGTCTGCACCTGTGACTTC, CTCCGTCTGTTAAACGCACA
CCR6A	XM_020110439	TCACGTCTGAGGTCTTCGTG TTGTGAGAGATGAGCCGTTG
IL-12A	XM_020101876	GGGGAGGATTTGCATCACTA TCTCCATGAGGGATTTGAGC
TLR1	XM_020081823.1	ACCGTCTCGCAGACTCTGTT ACGGAAACCTGGTTGTTCTG
β-ACTIN	XM_020109620	GGAATCCACGAGACCACCTA, AGCACAGTGTTGGCGTACAG

the RNA-seq protocol provided by Illumina and sequenced using an Illumina HiSeq 2500 system. Raw reads were filtered using the NGS QC Toolkit package to remove adaptor sequences and low-quality reads (base quality b < 20, read length b < 40 bp). The Trinity (r2013-11-10) pipeline program was used to assess and assemble contigs. Transcripts assembled from the total reads merged from each mRNA sample were mapped to the zebrafish genome together with the olive flounder genome using Tophat v2.0.4 with default parameters. Only those reads aligned against zebrafish or flounder genomes were considered in a differential gene expression analysis with Cuffdiff (Cufflinks v2.1.0 software) [42].

Differentially expressed genes and enriched gene ontology and pathway analysis

Transcript abundances in reads per kilobase per million reads mapped (RPKM) were estimated using RNA-Seq by expectation maximization (RSEM) through the Trinity plug-in, run_RSEM.pl. In order to identify the differential expression patterns of transcripts the TMM-normalized fragments per kilobase of transcript per million reads mapped (FPKM = total exon fragments/[mapped reads (millions)×exon length (kb)]) matrix was used for generating heat maps under an R programming environment. Functional annotations were conducted by comparing sequences with public databases. All Illumina-assembled unigenes were compared with the NCBI non-redundant protein database (http://www.ncbi.nlm.nih.gov/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg) using NCBI Blast (http://www.ncbi.nlm.nih.gov/). Gene Ontology (GO) terms were assigned to each unigene based on the GO terms annotated to its corresponding homolog. Unigenes were classified according to GO terms within molecular functions, cellular components, and biological processes. Further, Unigenes were assigned to special biochemical pathways according to the KEGG database using BLASTx, followed by the retrieval of KEGG Orthology (KO) information. The Search Tool for the Retrieval of Interacting Genes (STRING) v9.1 was used to examine protein–protein interaction networks [43].

Statistical analysis

Differences in the expression of innate immune response genes were evaluated by one-way ANOVA and two-way ANOVA using GraphPad Prism 5.0 (GraphPad Software, San Diego California USA, www.graphpad.com). A p value <0.05 was considered to indicate statistical significance.

Results

Growth of IHNV, VHSV, and HIRRV in HINAE cells

To determine whether IHNV grows in flounder cells as efficiently as VHSV and HIRRV, we compared infectious virus yields of IHNV, VHSV, and HIRRV in a flounder cell line, HINAE, at 20˚C. HINAE cells were infected with novirhabdovirus at 0.1 and 1 MOI and culture supernatants were collected at 1, 3, and 5 days after infection. The titers of infectious virus released into the media were determined by a plaque assay. At day 1 (24 h) p.i., although the titer of IHNV was 6.3~6.9-fold lower than that of VHSV, it was 1.6~2.1-fold higher than that of HIRRV (Fig 1). At day 3 and day 5 p.i., even though the titers of the three novirhabdoviruses showed a slight difference, the viral yield of all three significantly increased and the growth curve of IHNV was similar to those of VHSV and HIRRV (Fig 1). These results suggest that IHNV is competent to replicate in flounder cells as VHSV and HIRRV.
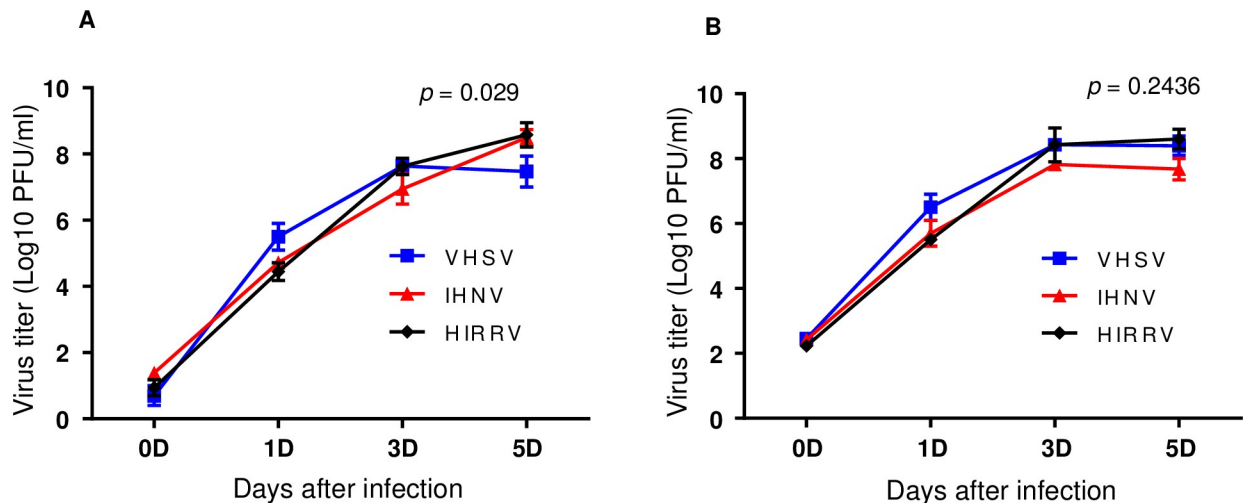
Fig 1. Growth kinetics of IHNV, VHSV, and HIRRV in HINAE cells. The cells were infected at a multiplicity of infection of (A) 0.1 or (B) 1 plaque-forming unit (PFU) per cell and samples of the supernatant medium were collected at the indicated time points. Viral titers were determined in duplicate by plaque assay on epithelioma papulosum cyprini (EPC) cells. Data are mean ± SD (n = 3). Two-way ANOVA. *p* values are indicated in the graphs.

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Sequencing and de novo assembly

To search for the genome-wide cell response to novirhabdovirus infection, we analyzed the transcriptomes of HINAE cells infected with IHNV, VHSV, and HIRRV by using RNA-seq and compared them with mock-treated cells. We obtained 7.1, 6.7, 8.3, and 7.2 gigabase (GB) of raw data from mock, VHSV-, IHNV-, and HIRRV-infected HINAE cells, respectively, by paired-end sequencing. After the removal of *Illumina* adaptors and filter sequences, a total of 184,875,094 cleaned reads were obtained from mock and virus-infected HINAE cells. These high-quality reads were *de novo* assembled using Trinity software v.2.2.0 [44]. Detailed information on the *de novo* transcriptome assembly is summarized in Table 3. The prediction of coding regions in the assembled transcripts was carried out using TransDecoder (implemented in Trinity software). In total, 59,980 trinity genes were found. The N50 contig length was 2,105 bp and median contig length and average contig were 427 bp and 965 bp, respectively (Table 3).

Table 3. Summary of Illumina transcriptome sequencing and assembly for HINAE cells.

Summary of raw read data	Total number of raw reads	186,432,802
	Total number of clean reads	184,875,094
	Average read length after filtering (bp)	100
	Sequence quality ≥ Q30 (%)	92.99
	GC%	50.66
Summary of the assembled transcriptome	Total trinity 'genes'	180,292
	Total trinity transcripts (bp)	302,256
	GC%	47.17
	Contig N50 (bp)	2,105
	Median contig length (bp)	427
	Average contig (bp)	965
	Total assembled bases	121,968,120

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Differential expression, functional annotation, and functional enrichment analysis

A total of 21,207, 20,994, and 21,335 differentially expressed genes (DEGs) were detected in IHNV-, VHSV-, and HIRRV-infected HINAE cells, respectively, when compared to mock-infected HINAE cells (S1–S3 Tables). By using gene set enrichment analysis (GSEA) [45], we identified 12,538, 12,466, and 12,595 unigenes from DEGs detected in IHNV-, VHSV-, and HIRRV-infected HINAE cells, respectively. We next conducted GO enrichment analysis to identify the major gene groups affected by infection with IHNV, VHSV, and HIRRV. GO enrichment analysis assigned these unigenes to three categories (biological process, cellular component, and molecular function) and subsequently categorized them into 53 functional groups (Fig 2 and S4 Table). Even though there was a slight difference in the number of genes in each functional group, unigenes from IHNV-, VHSV-, and HIRRV-infected HINAE cells showed a similar distribution pattern throughout the 53 functional groups (Fig 2 and S4 Table). The most prevalent GO terms were metabolic process, biological regulation, and response to stimulus in the biological process category; membrane, nucleus, and macromolecular complex in the cellular component category; and ion binding, nucleic acid binding, and protein binding in the molecular function category.

To better understand the biological functions of the unigenes, we mapped them to the referential canonical pathways in the KEGG database. The top 10 pathways based on FDR values for up- and down-regulated unigenes per each virus-infected cell are presented in Table 4. Unigenes could be mapped to 55 KEGG pathways, indicating that novirhabdovirus infection modulates the expression of cellular genes involved in a broad variety of pathways. The
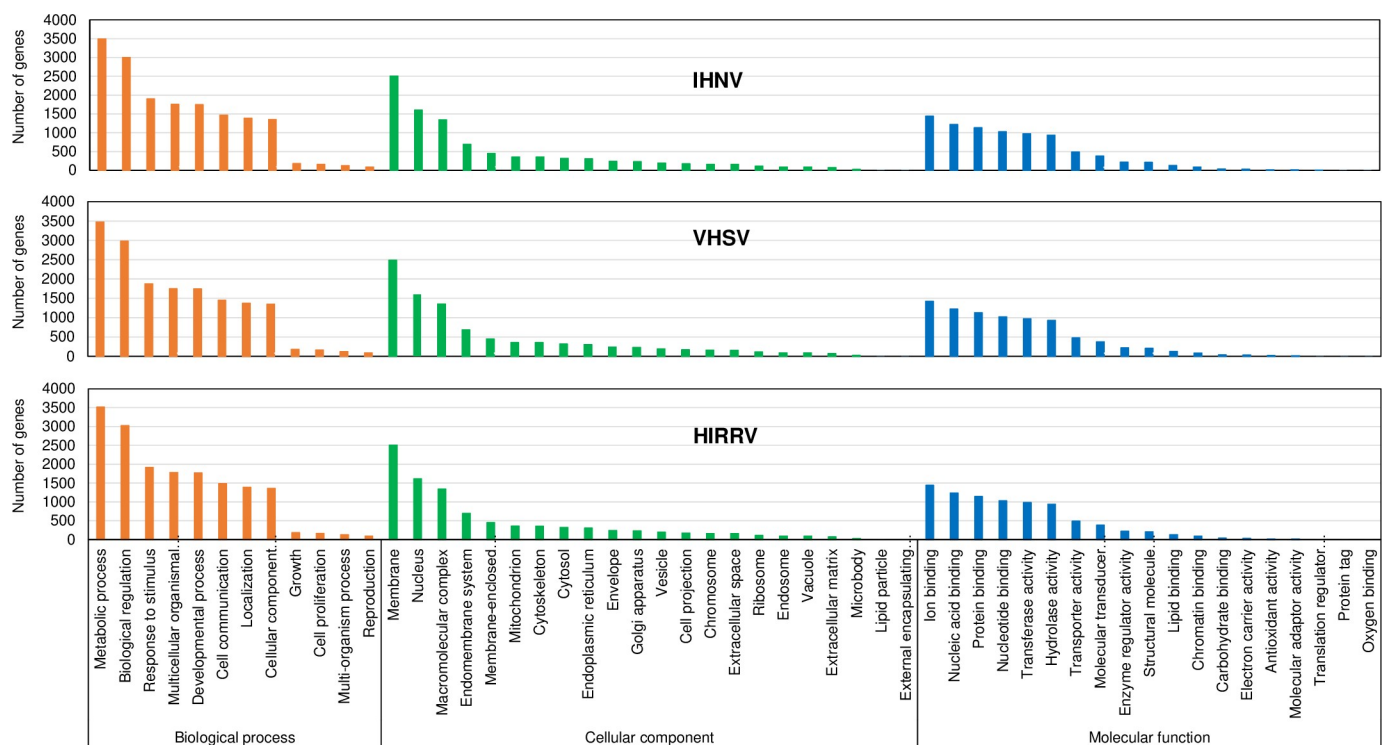
Fig 2. Gene Ontology (GO) categories of the unigenes. Unigenes from IHNV-, VHSV-, and HIRRV-infected HINAE cells were annotated to the 53 functional GO terms in three categories: cellular components, molecular functions, and biological process. S4 Table contains detailed information about number of genes annotated to the 53 functional GO terms.

Table 4. KEGG pathway analysis of unigenes of IHNV-, VHSV-, and HIRRV-infected HINAE cells.

| Virus	Up-regulated pathways			Down-regulated pathways		
	KEGG pathway name	FDR	No. of genes	KEGG pathway name	FDR	No. of genes
IHNV	Notch signaling pathway	7.78E-01	44	Ribosome	9.59E-03	118
	Other types of O-glycan biosynthesis	8.01E-01	22	Oxidative phosphorylation	1.09E-01	86
	Glycosaminoglycan degradation	8.26E-01	14	Ascorbate and aldarate metabolism	1.13E-01	10
	Primary bile acid biosynthesis	8.56E-01	9	Folate biosynthesis	1.24E-01	13
	Autophagy	8.82E-01	23	Porphyrin and chlorophyll metabolism	5.58E-01	21
	Progesterone-mediated oocyte maturation	8.97E-01	78	Phototransduction	8.85E-01	20
	Glycosaminoglycan biosynthesis—keratan sulfate	9.17E-01	11	Mucin type O-glycan biosynthesis	9.16E-01	18
	Fatty acid elongation	9.23E-01	19	Spliceosome	9.31E-01	110
	Phenylalanine metabolism	9.25E-01	11	ABC transporters	9.35E-01	32
	N-Glycan biosynthesis	9.33E-01	41	Pantothenate and CoA biosynthesis	9.64E-01	11
VHSV	Neuroactive ligand-receptor interaction	8.63E-03	179	Ribosome biogenesis in eukaryotes	0E+00	61
	Cell adhesion molecules	1.04E-01	75	Spliceosome	0E+00	110
	Drug metabolism—other enzymes	1.98E-01	20	DNA replication	9.95E-04	32
	Calcium signaling pathway	2.02E-01	158	Glycosylphosphatidylinositol-anchor biosynthesis	1.24E-03	20
	Cytokine-cytokine receptor interaction	2.13E-01	96	Nucleotide excision repair	1.52E-03	42
	Adrenergic signaling in cardiomyocytes	2.20E-01	128	Base excision repair	1.66E-03	29
	Tryptophan metabolism	2.33E-01	32	Basal transcription factors	2.52E-03	37
	ABC transporters	2.42E-01	34	Fanconi anemia pathway	2.80E-03	44
	Ether lipid metabolism	2.43E-01	26	Peroxisome	6.13E-03	67
	Tyrosine metabolism	2.49E-01	22	RNA transport	7.97E-03	132
HIRRV	Taurine and hypotaurine metabolism	9.78E-01	6	Ribosome	3.47E-01	118
	RNA polymerase	9.89E-01	29	Folate biosynthesis	5.37E-01	13
	Protein export	9.97E-01	19	Arachidonic acid metabolism	6.23E-01	28
	Purine metabolism	9.97E-01	140	Nicotinate and nicotinamide metabolism	7.38E-01	22
	Insulin signaling pathway	9.98E-01	124	Mismatch repair	9.69E-01	21
	Herpes simplex infection	9.99E-01	124	Protein processing in endoplasmic reticulum	9.97E-01	134
	Progesterone-mediated oocyte maturation	1.00E+00	79	Oxidative phosphorylation	1.00E+00	86
	Oocyte meiosis	1.00E+00	96	Tight junction	1.00E+00	113
	Adherens junction	1.00E+00	65	Neuroactive ligand-receptor interaction	1.00E+00	181
	FoxO signaling pathway	1.00E+00	131	Pantothenate and CoA biosynthesis	1.00E+00	11

distribution of gene pathways among unigenes from IHNV-, VHSV-, and HIRRV-infected cells was quite different. However, genes related to signaling pathways were commonly up-regulated but those related to ribosome and repair were commonly down-regulated. This analysis showed enriched pathways in flounder cells associated with novirhabdovirus infection.

Modulation of the innate immune response by the three novirhabdoviruses

The growth of a virus in host cells depends on its ability to modulate the innate immune response in infected cells [35–37]. We found that IHNV grew as well as VHSV and HIRRV in flounder cells, suggesting that IHNV can modulate the key innate immune response of flounder cells as efficiently as VHSV and HIRRV. To determine the innate immune responses of flounder cells which are commonly modulated by three novirhabdoviruses, we analyzed the expression changes in innate immune response genes of HINAE cells infected with IHNV, VHSV, and HIRRV for 24 h and compared them with mock-infected control cells.

From the RNA-seq analysis, we identified 170 DEGs involved in innate immune response at 24 h p.i. (Fig 3A and S5 Table). Of the 170 DEGs, 22, 66, and 28 genes showed more than a
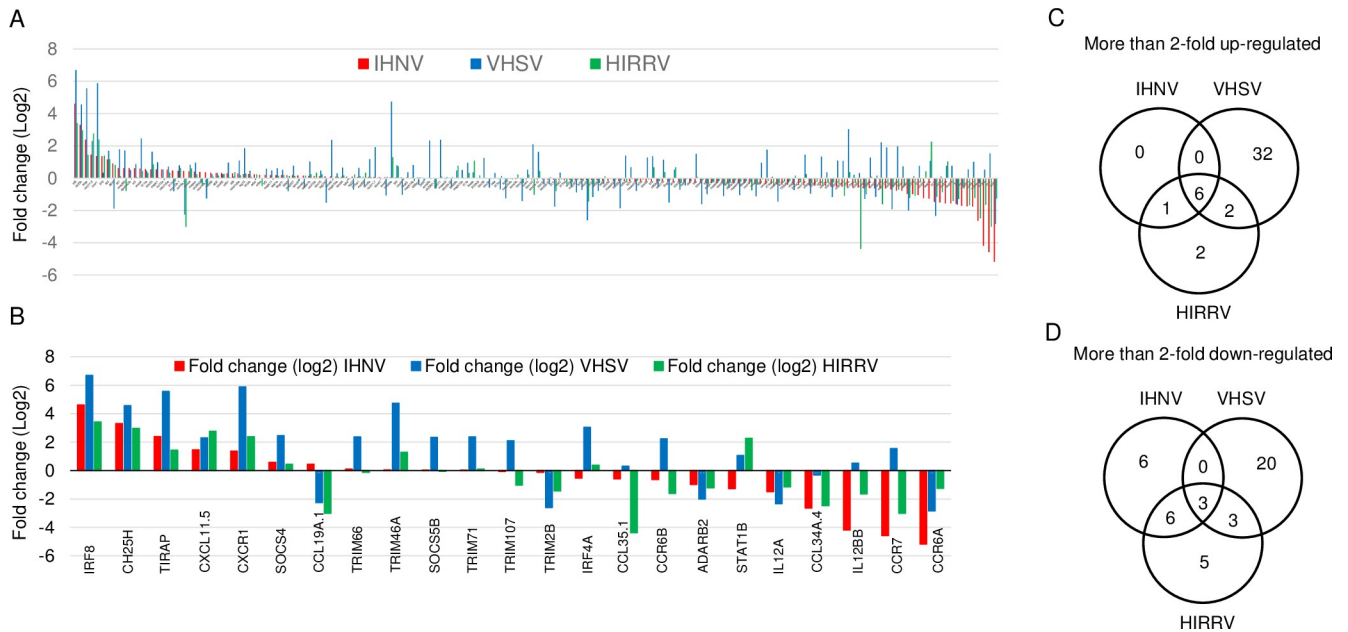
Fig 3. Effect of novirhabdovirus infection on host gene expression of HINAE cells. (A) Fold changes of (A) 170 innate immune response genes and (B) 23 innate immune response genes with more than 4-fold change in HINAE cells at 24 h after 1 multiplicity of infection (MOI) with IHNV, VHSV, or HIRRV. S5 Table contains detailed information about gene name and fold change value for each gene. (C & D) Numbers in the Venn diagrams (http://bioinfogp.cnb. csic.es/tools/venny/index.html) represent the number of cellular genes with more than two-fold (C) up-regulated and (D) down-regulated in IHNV-, VHSV- , and HIRRV-infected cells at 24 h post-infection.

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2-fold expression change in HINAE cells after infection with IHNV, VHSV, and HIRRV, respectively (Fig 3A and S5 Table). In particular, 9 genes were commonly modulated with more than a 2-fold change by the three novirhabdoviruses: 6 genes, interferon regulatory factor 8 (*IRF8*), C-X-C motif chemokine receptor 1 (*CXCR1*), TIR domain containing adaptor protein (*TIRAP*), cholesterol 25-hydroxylase (*CH25H*), chemokine (C-X-C motif) ligand 11, duplicate 5 (*CXCL11.5*), and Toll-like receptor 2 (*TLR2*), were increased; 3 genes, chemokine (C-C motif) receptor 6a (*CCR6a*), interleukin 12a (*IL12a*), and Toll-like receptor 1 (*TLR1*), were decreased (Fig 3B and 3C, and Table 5). These results suggest that modulation of these genes may be essential for the replication of the three novirhabdoviruses in flounder cells. On the other hand, 65 genes were species-specific: 6 IHNV-specific genes, 52 VHSV-specific genes, and 7 HIRRV-specific genes (Fig 3B and 3C, and Table 5). These species-specific genes may be involved in the species-specific pathogenicity of the novirhabdoviruses.

To confirm these results, we selected 9 commonly modulated genes and determined by qRT-PCR their expression levels in novirhabdovirus-infected HINAE cells. Consistent with RNA-seq results, we found a significant increase in gene expression for *IRF8*, *CXCR1*, *TIRAP*, *CH25H*, *CXCL11.5*, and *TLR2* and a significant decrease in gene expression for *CCR6a*, *IL-12A*, and *TLR1* at 24 h after infection with the three novirhabdoviruses (Fig 4 and S6 Table). These results suggest that the induction of these genes is a common event in HINAE cells infected by IHNV, VHSV, and HIRRV.

Protein–protein interaction network analysis

The biological associations among the innate immune response genes modulated by IHNV, VHSV, and HIRRV with more than 2-fold change were investigated using STRING software. The predicted protein–protein associations were queried through a vast number of databases

Table 5. Genes modulated more than 2-fold in HINAE cells at 24 h after infection with IHNV, VHSV, or HIRRV.

	Virus specificity	Gene name
>2-fold increase	Common to IHNV, VHSV, and HIRRV	IRF8, CXCR1, TIRAP, CH25H, CXCL11.5, TLR2
	IHNV	-*
	VHSV	IRF4A, SOCS4, TRIM71, TRIM66, SOCS5B, CCR6B, TRIM107, TRIM46B, CXCL11.7, MAPK12B, ISG15, TLR7, CXCR4A, CASP8L2, TRIM25, TRIM35-12, CCR7, IRF3, MOV10A, CRFB2, IL1B, IRF1A, TRIM8B, TRIM62, TRIM36, TRIM8A, TRIM110, TRIM24, MHC1UBA, CCL20A.3, ADARB1B, CCL36.1
	HIRRV	FOSAB, CXCL19
>2-fold decrease	Common to IHNV, VHSV, and HIRRV	CCR6A, IL12A, TLR1
	IHNV	CXCR5, CXCL19, CCR10, STAT1B, IL12RB2, IL10
	VHSV	CXCL14, IFIT16, TRIM55A, IL17C, TRIM45, ISG20L2, IFNGR1, RNASEH1, TRIM32, SOCS7, MOV10B.2, TLR3, TRIM2A, TRIM105, SOCS1A, SOCS2, TRIM33L, SOCS9, RNASEH2B, CDC40
	HIRRV	CCL35.1, CCR6B, CCL20A.3, TRIM109, TRIM107

IRF8, interferon response factor 8; CXCR1, C-X-C motif chemokine receptor 1; TIRAP, TIR domain containing adaptor protein; CH25H, cholesterol 25-hydroxylase; CXCL11.5, chemokine (C-X-C motif) ligand 11, duplicate 5; TLR2, Toll-like receptor 2; IRF4A, interferon response factor 4a; SOCS4, Suppressor Of Cytokine Signaling 4; TRIM71, Tripartite Motif Containing 71; TRIM66, Tripartite Motif Containing 71; SOCS5B, Suppressor Of Cytokine Signaling 5b; CCR6B, chemokine (C-C motif) receptor 6b; TRIM107, Tripartite Motif Containing 107; TRIM46B, Tripartite Motif Containing 46b; CXCL11.7, chemokine (C-X-C motif) ligand 11, duplicate 7; MAPK12B, mitogen-activated protein kinase 12b; ISG15, interferon-stimulated gene 15; TLR7, Toll-like receptor 7; CXCR4A, chemokine (C-X-C motif) receptor 4a; CASP8L2, caspase 8, apoptosis-related cysteine peptidase, like 2; TRIM25, Tripartite Motif Containing 25; TRIM35-12, Tripartite Motif Containing 71; CCR7, chemokine (C-C motif) receptor 7; IRF3, interferon response factor 3; MOV10A, Moloney leukemia virus 10a; CRFB2, cytokine receptor family member B2; IL1B, interleukin 1b; IRF1A, interferon response factor 1a; TRIM8B, Tripartite Motif Containing 8b; TRIM62, Tripartite Motif Containing 62; TRIM36, Tripartite Motif Containing 36; TRIM8A, Tripartite Motif Containing 8a; TRIM110, Tripartite Motif Containing 110; TRIM24, Tripartite Motif Containing 24; MHC1UBA, Major histocompatibility complex class I UBA; CCL20A.3, chemokine (C-C motif) ligand 20a, duplicate 3; ADARB1B, adenosine deaminase RNA specific B1b; CCL36.1, chemokine (C-C motif) ligand 36, duplicate 1; FOSAB, v-fos FBJ murine osteosarcoma viral oncogene homolog Ab; CXCL19, chemokine (C-X-C motif) ligand 19; CCR6A, chemokine (C-C motif) receptor 6a; IL12A, interleukin 12a; TLR1, Toll-like receptor 1; CXCR5, chemokine (C-X-C motif) receptor 4a; CCR10, chemokine (C-C motif) receptor 10; STAT1B, Signal transducer and activator of transcription 1b; IL12RB2, interleukin 12 receptor subunit beta 2; IL10, interleukin 10; CXCL14, chemokine (C-X-C motif) ligand 14; IFIT16, interferon-induced protein with tetratricopeptide repeats 16; TRIM55A, Tripartite Motif Containing 55a; IL17C, interleukin 17c; TRIM45, Tripartite Motif Containing 45; ISG20L2, interferon stimulated exonuclease gene 20 like 2; IFNGR1, Interferon gamma receptor 1; RNASEH1, Ribonuclease H1; TRIM32, Tripartite Motif Containing 32; SOCS7, Suppressor Of Cytokine Signaling 7; MOV10B.2, Moloney leukemia virus 10b, tandem duplicate 2; TLR3, Toll-like receptor 3; TRIM2A, Tripartite Motif Containing 2a; TRIM105, Tripartite Motif Containing 105; SOCS1A, Suppressor Of Cytokine Signaling 1a; SOCS2, Suppressor Of Cytokine Signaling 2; TRIM33L, Tripartite motif-containing 33,-like; SOCS9, Suppressor Of Cytokine Signaling 9; RNASEH2B, Ribonuclease H2, subunit B; CDC40, Cell Division Cycle 40; CCL35.1, chemokine (C-C motif) ligand 35, duplicate 1; TRIM109, Tripartite Motif Containing 109.

*, no genes specific to IHNV.

derived in different ways (e.g. experimentally determined interactions, protein neighborhood data, or data acquired via text mining). Interestingly, most of the innate immune response genes modulated by the three novirhabdoviruses were found to be connected with *TLR1/ TLR2/TIRAP* genes and *CXCR1/CCR6a* genes (Fig 5).
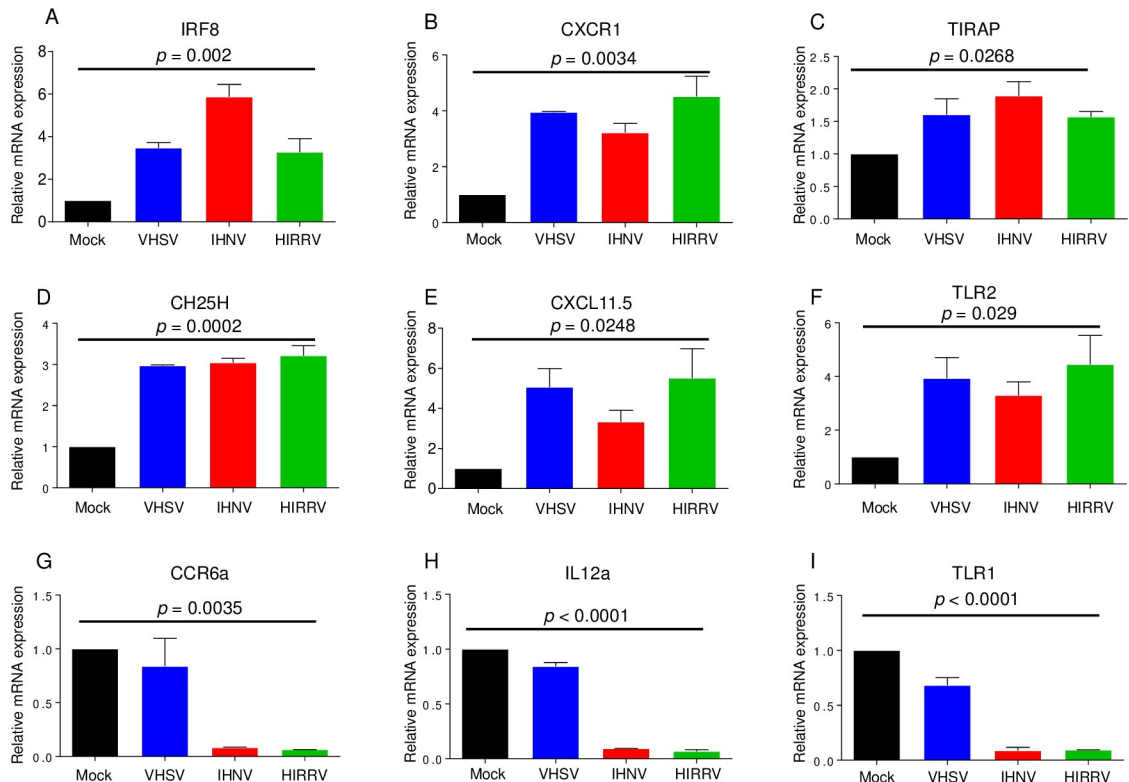
Fig 4. Confirmation of RNA-seq data by RT-qPCR after novirhabdovirus infection. Gene expression for the indicated genes was assessed by qRT-PCR from HINAE cells after 1 multiplicity of infection (MOI) with IHNV, VHSV, or HIRRV at 20˚C for 12 h and 24 h. The *β*-actin gene was used as a reference to normalize the data. Expression values are shown as fold change compared to untreated cells. Data are mean ± SEM (*n* = 6). One-way ANOVA. *p* values are indicated in the graphs. S6 Table contains detailed information about Ct values of each gene.

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Discussion

In this study, we first investigated whether IHNV is competent to replicate in flounder cells as VHSV and HIRRV. We compared the production of infectious viral progeny by the three novirhabdoviruses in flounder cells and found that the production of IHNV was similar to that of VHSV and HIRRV. These results suggest that IHNV is competent to replicate in flounder cells.

Novirhabdovirus infection induces a rapid IFN and ISG response [46–49] and the pre-activated IFN and ISG response inhibits the replication of novirhabdoviruses [50–53]. For successful replication within host cells, novirhabdovirus must possess a mechanism to inhibit the IFN response of host cells. To better understand the underlying mechanisms of the IHNV-mediated modulation of IFN responses in flounder cells, we compared the whole genome transcriptome analysis of flounder cells infected with three novirhabdoviruses at 24 h p.i. using RNA-seq. We first determined the cellular transcriptome profile to investigate the changes in host expression during infection. Infection by the novirhabdoviruses induced massive changes (about 12,500 unigenes compared to the transcriptome of uninfected control cells) in the flounder cell transcriptome. GO enrichment analysis revealed that about 12,500 unigenes from novirhabdovirus-infected flounder cells could be assigned to 53 functional groups and that unigenes from IHNV-, VHSV-, and HIRRV-infected cells similarly distributed to these 53 functional groups. GO enrichment analysis also suggest that HINAE transcriptome is similarly
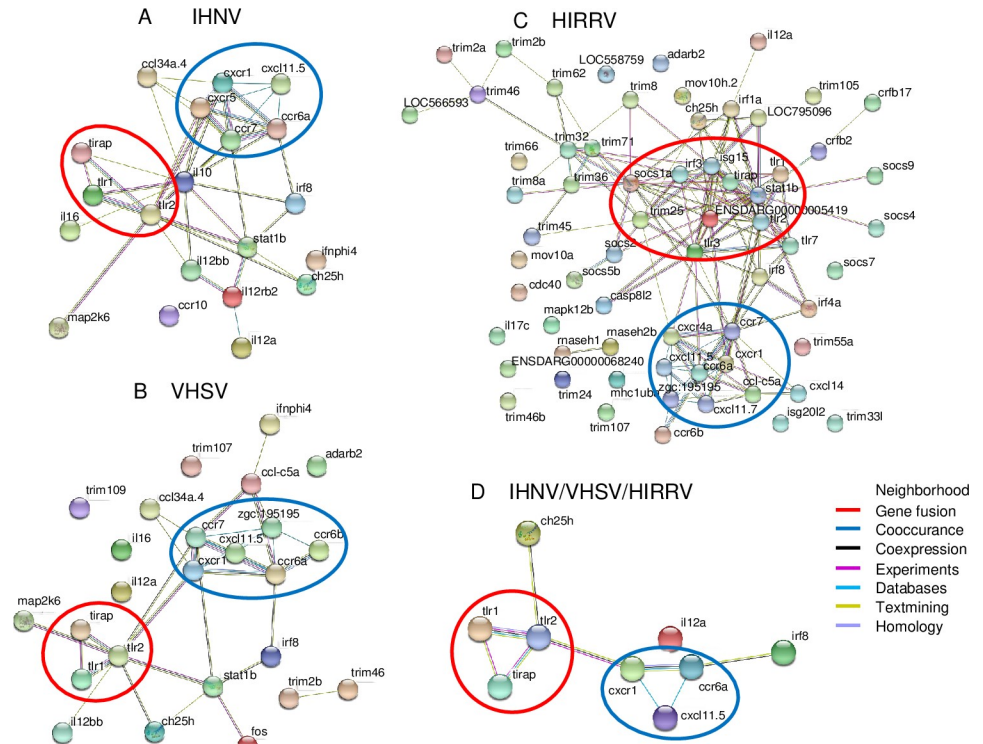
Fig 5. Interaction networks of innate immune response genes modulated by novirhabdovirus infection by STRING analysis. Interaction networks of the associated proteins were found among 22, 66, and 28 genes with more than 2-fold expression change in HINAE cells after infection with (A) IHNV, (B) VHSV, and (C) HIRRV, respectively. (D) Protein interaction network analysis of 9 genes commonly modulated by IHNV, VHSV, and HIRRV. The interaction networks included two highly connected protein nodes: red circle, node consisted of TLR1/TLR2/TIRAP proteins; blue circle, node consisted of CXCR1/CCR6a proteins. Different line colors represent the types of evidence for the association.

modulated by the three novirhabdoviruses and that a large part of the modulated HINAE transcriptome is devoted to the control of the main metabolic processes and to binding (ion, nucleic acid, and protein). Contrary to the result of GO enrichment analysis, unigenes from the three novirhabdoviruses quite differently distributed to 55 KEGG pathways. Even though the distribution pattern of the unigenes affected by the three novirhabdoviruses was different we could detect a common trend: genes related to signaling pathway were up-regulated and those related to ribosome and repair were down-regulated.

In HINAE cells, we found that novirhabdovirus infection altered the expression of 170 genes related to the IFN response at 24 h p.i. Among 170 genes, 6 genes (*IRF8*, *CXCR1*, *TIRAP*, *CH25H*, *CXCL11.5*, and *TLR2*) were up-regulated more than two-fold by three novirhabdoviruses. In particular, there was a 103-fold, 24-fold, and 10-fold increase of *IRF8* expression in VHSV-, IHNV-, and HIRRV-infected cells, respectively. *IRF8*, a transcription factor, is able to either inhibit [54, 55] or induce IFN gene transcription [56–58] depending on cell type and conditions. The role of IRF8 in antiviral immunity also depends on cell type and condition. For example, IRF8 in cluster of differentiation 8$^+$ (CD8$^+$) T cells suppresses the activation and expansion of CD8$^+$ T cells in herpes simplex virus 1 (HSV-1) infected mice, which reduces antiviral immunity [59]. On the other hand, IRF8 in human monocytes is involved in the rapid induction of IFN-β after exposure to Sendai virus [57]. In addition, IRF8 in mouse natural killer cells exposed to cytomegalovirus facilitates the proliferation of virus-specific natural

killer (NK) cells and enhances NK cell mediated antiviral immunity [60]. Even though it has been reported that *IRF8* is induced by viruses in olive flounder [61], the role of *IRF8* in antiviral immunity in fish species has not yet been determined.

As in *IRF8*, the other genes in the group of six that were up-regulated, *CXCR1*, *TIRAP*, *CH25H*, *CXCL11.5*, and *TLR2*, have been reported to be induced by virus infection and have both antiviral and proviral functions. CXCR1, a member of the G-protein-coupled receptor family is a chemotactic factor [62]. CXCR1 and its ligand C-X-C motif chemokine ligand 8 (CXCL8)/interleukin 8 (IL-8) have been reported in multiple fish species [63] and fish CXCL8/IL-8 is also induced in spleen upon infection with VHSV [64] and IHNV [46]. Co-injection of IL-8 with VHSV G-encoding DNA vaccine in rainbow trout enhanced the production of proinflammatory cytokine [65]. This suggests the possibility that CXCR1 may have antiviral function by enhancing recruitment of immune cells to the virus infection site. However, there are several reports indicating that CXCR1 has proviral function. CXCR1 ligand IL-8 induced by virus infection enhances the replication of several viruses including encephalomyocarditis virus (EMCV) [66] and cytomegalovirus (CMV) [67].

TIRAP is an adaptor protein in the TLR signaling pathway that functions downstream of TLRs [68, 69]. Recruitment of TIRAP to TLRs induces antiviral innate immune response through the activation of nuclear factor (NF)-κB and IRFs. For example, upon stimulation by herpes simplex virus (HSV) and influenza viruses, TIRAP in association with TLR9 and TLR7 induces expression of IFN-α which plays an antiviral role [70]. In macrophages after exposure to HSV-1, TIRAP is involved in the induction of IL-15 [71], which is essential for the generation, activation, and proliferation of NK and natural killer T (NKT) cells [72]. On the contrary, it has been reported that NF-κB downstream of TIRAP can enhance HSV-1 gene expression and increase viral yields [73].

CH25H is rapidly induced by various TLR ligands and IFN molecules [74]. CH25H catalyzes the formation of 25-hydroxycholesterol (25HC) which exerts an antiviral activity through both 25HC-dependent and independent events. The 25HC protein impairs viral entry at the virus-cell membrane fusion step by inducing cellular membrane changes [75] and, thus, suppresses infection of enveloped viruses including vesicular stomatitis virus (VSV) and human immunodeficiency virus type 1 (HIV-1) [75, 76]. Alternatively, CH25H has been reported to interact with the nonstructural protein 5A (NS5A) protein of hepatitis C virus (HCV) and inhibit HCV replication [77]. In zebrafish, CH25H is induced by Spring Viremia Carp Virus (SVCV), inhibits VHSV replication, and protects zebrafish larvae from SVCV infection [78]. In addition, CH25H is induced by Singapore grouper iridovirus (SGIV) and red-spotted grouper nervous necrosis virus (RGNNV) in grouper cells and inhibits the entry and replication of these viruses [79].

TLR2 is a member of the TLR family. TLR2 stimulated by ligand activates NF-κB to induce production of cytokines, including IFN-β and inflammatory cytokines, and chemokines [80]. The major TLR2 ligands are lipoproteins, highly expressed in the outer membrane of Gram-positive bacteria [81]. TLR2 can recognize viral components such as envelope glycoproteins [82]. Evidence suggests that TLR2 plays antiviral roles: TLR2 stimulated by vaccinia virus and murine CMV induces IFN-β production in monocytes [83]; TLR2 knockout impairs NK cell activity and increases the CMV load in mice [84]. In fish cells, TLR2 expression shows a negative correlation with the transcription level of VHSV in the kidney of olive flounder [85]. In turbot, VHSV infection significantly down-regulates vaccine-induced TLR2 expression [86]. By contrast with these findings, in this study, we found that the infection of novirhabdoviruses increased the expression of *TLR2* in flounder cells.

In this study, we found that *IRF8*, *CXCR1*, *TIRAP*, *CH25H*, *CXCL11.5*, and *TLR2* are commonly induced by three novirhabdoviruses in flounder cells, indicating that the upregulation

of these six genes is essential in novirhabdovirus-infected flounder cells. It is highly possible that these six genes may have an antiviral function in other virus-infected cells and play a role in the suppression of viral growth. However, it is also possible that these genes could mediate signaling pathways that may be required for the efficient growth of novirhabdoviruses in flounder cells. More work should be done to determine whether these six genes induced by the three novirhabdoviruses in flounder cells play an antiviral role or support the growth of novirhabdovirus in flounder cells.

On the contrary, the expression of three innate immune response genes, including *CCR6a*, *IL12a*, and *TLR1* was down-regulated more than two-fold by three novirhabdoviruses. CCR6a is a CC chemokine receptor found in teleost fish [87, 88]. It has been reported that CCR6a is induced in olive flounder after infection with *Vibrio anguillarium* [88], indicating that CCR6a plays a role in the immune response against infection. However, its role in infection has not yet been determined. CCR6a in teleost fish shows the highest identity with human and mouse CCR6 [87]. C-C motif chemokine ligand 20 (CCL20) is the only confirmed chemokine ligand of CCR6 [89]. CCR6 is induced by virus infection and modulates the migration of immune cells. For example, Kaposi's sarcoma-associated herpesvirus (KSHV) induces CCR6 expression in human umbilical vein endothelial cells, which may contribute to the recruitment of dendritic cells (DCs) and lymphocytes into the Kaposi's sarcoma lesion [90]. Migration of immune cells induced by CCR6 and CCL20 signaling may be beneficial to viral growth since neutralization of CCL20 or CCR6 depletion decreases the migration of conventional DCs to the lung and enhances clearance of respiratory syncytial virus (RSV) [91].

IL12a is a subunit of the cytokine IL-12, a heterodimer composed of IL-12a and IL-12b. IL-12 is a proinflammatory cytokine that promotes the differentiation of type 1 T helper (Th1) cells. Accumulating evidence suggests that IL-12 plays a role in the efficient immune response to virus infections. It enhances the production of antiviral cytokines and amplifies the cytotoxicity of cytotoxic T lymphocytes [92] which can kill hepatitis B virus (HBV)-infected hepatocytes [93]. IL-12 induces the production of TNF-α and INFα/ß which inhibits the growth of hepatitis B virus in liver and kidney cells [94]. To escape from the immune response, HBV down-regulates IL-12a expression after infection into host cells [95].

TLR1 in association with TLR2 can recognize envelope glycoprotein gB and gH of human cytomegalovirus (HCMV) [96]. In addition, *TLR1* expression is induced by influenza A virus and Sendai virus in human macrophages [97]. This suggests that TLR1 has an antiviral role. TLR1 has been identified in olive flounder [98]. In addition, TLR1 expression is induced by VHSV infection in fish cells and may play an antiviral role [99]. Here we found that the expression of *CCR6a*, *IL12a*, and *TLR1* was down-regulated by the three novirhabdoviruses in flounder cells. When considering the antiviral function of these genes, their inhibition seems to be essential for the efficient growth of novirhabdoviruses in flounder cells. However, the role of these genes in innate immunity against novirhabdovirus infection in fish cells remains to be determined.

Beside the above-mentioned common genes, our results indicated that three novirhabdoviruses affected different sets of innate immune response genes in cells. Of the 170 innate immune response genes, VHSV infection affected the expression of 66 genes more than two-fold, while IHNV and HIRRV altered the expression of 22 and 28 genes more than two-fold, respectively. These indicate that flounder cells respond more actively to VHSV infection than to IHNV or HIRRV. However, these differences in innate immune responses might not be critical for the regulation of viral growth in cells since three novirhabdoviruses showed similar proliferation in cells. It is well known that innate immune responses play a crucial part in the initiation and subsequent direction of adaptive immune response [100]. Thus, the differences in the expression of innate immune response genes by three novirhabdoviruses may affect the

efficiency of differentiation of appropriate adaptive immune responses required for protection against novirhabdoviruses and thus would have a connection to viral pathogenesis. Since both VHSV and HIRRV are pathogenic to flounder, the number of innate immune response genes altered by novirhabdovirus infection may not be critical to determine novirhabdovirus pathogenesis. Instead, specific genes/pathways affected by novirhabdovirus infection would determine viral pathogenesis. In this context, two groups of genes would play a critical role for connection to novirhabdovirus pathogenesis: 5 genes (2 genes, *TRIM46a* and *STAT1b*, up-regulated; 3 genes, *ADARb2*, *CCL19a.1*, and *TRIM2b*, down-regulated) which are commonly modulated more than two-fold by pathogenic both VHSV and HIRRV but not by IHNV; 6 genes (*IL10*, *IL12Rb2*, *STAT1b*, *CCR10*, *CXCL19*, and *CXCR5*) which are down-regulated more than two-fold only by IHNV. In addition, even though both VHSV and HIRRV are pathogenic to flounder, the overall innate immune response genes affected by these two viruses were distinct from each other, suggesting that their pathogenic mechanisms and disease symptom would differ between them. Further studies on the role of these genes in novirhabdovirus infection may reveal why IHNV is non-pathogenic in flounder while it proliferates well in host cells.

In summary, we show that IHNV is competent to replicate in flounder cells, similar to VHSV and HIRRV, even though it cannot cause disease in flounders. RNA-seq analysis identified 6 (*IRF8*, *CXCR1*, *TIRAP*, *CH25H*, *CXCL11.5*, and *TLR2*) and 3 (*CCR6a*, *IL-12a*, and *TLR1*) innate immune response genes commonly up-regulated and down-regulated in flounder cells, respectively, by all three novirhabdoviruses. Even though their antiviral activities against novirhabdovirus infection have not been determined, these genes, individually or collectively, may have important roles in the restriction of novirhabdovirus infection. However, our results suggest that the ability of novirhabdovirus to modulate the expression of these genes in virus-infected cells may not be the critical determinant for novirhabdovirus virulence in flounder. Consistently, it has been reported that IHNV virulence does not correlate with inhibition of IFN response in IHNV-infected fish [47].

Previously, many genome-wide analyses have been conducted to determine immune response to novirhabdoviruses in olive flounder [86, 99, 101–107], rainbow trout (*Oncorhynchus mykiss*) [108], sockeye salmon (*Oncorhynchus nerka*) [109], and turbot (*Scophthalmus maximus*) [86] and identified a large number of immune response genes modulated by novirhabdovirus infection. Of the 9 genes commonly modulated by three novirhabdoviruses, consistent with our data, genome-wide analyses identified TLR1 [93] and TLR2 [80] as being modulated by VHSV infection. However, we could not detect the change in the expression of the other 7 genes from the previous genome-wide analyses of novirhabdovirus-infected fish. While we used a flounder cell line HINAE, most other studies used tissue samples containing heterogeneous cells from virus-infected fish. Thus, it is possible that the previous genome-wide analyses might preferentially detect innate immune responses exerted by immune cells within the tissues rather than by virus-infected cells.

Beside the innate immune response, adaptive immune responses such as neutralizing antibody-mediated humoral immune response and cell-mediated immune response play important roles in protecting fish from novirhabdovirus infection [33]. Further studies for the comparison of transcriptome changes in flounder infected by IHNV, VHSV, and HIRRV are required to understand why–despite a robust innate immune response–flounder cell lines fail to shut down the replication of IHNV and why IHNV is avirulent for flounder.

Supporting information

S1 Table. List of differentially expressed genes (DEGs) in mock- and IHNV-infected HINAE cells (mock vs IHNV).
(XLSX)

S2 Table. List of DEGs in mock- and VHSV-infected HINAE cells (mock vs VHSV).
(XLSX)

S3 Table. List of DEGs in mock- and HIRRV-infected HINAE cells (mock vs HIRRV).
(XLSX)

S4 Table. Annotation of unigenes from IHNV-, VHSV-, HIRRV-infected HINAE cells to functional GO terms.
(XLSX)

S5 Table. List of 170 DEGs involved in innate immune response in IHNV-, VHSV-, or HIRRV-infected HINAE cells (mock vs virus).
(XLSX)

S6 Table. Cycle threshold (Ct) values used to generate graphs in Fig 4.
(XLSX)

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References

1. Kurath G, Leong JC. Characterization of infectious hematopoietic necrosis virus mRNA species reveals a nonvirion rhabdovirus protein. Journal of virology. 1985; 53(2):462–8. https://doi.org/10.1128/JVI.53.2.462-468.1985 PMID: 4038520

2. Johnson MC, Simon BE, Kim CH, Leong J-AC. Production of recombinant snakehead rhabdovirus: the NV protein is not required for viral replication. Journal of virology. 2000; 74(5):2343–50. https://doi.org/10.1128/jvi.74.5.2343-2350.2000 PMID: 10666265

3. Schütze H, Enzmann P-J, Kuchling R, Mundt E, Niemann H, Mettenleiter T. Complete genomic sequence of the fish rhabdovirus infectious haematopoietic necrosis virus. Journal of General Virology. 1995; 76(10):2519–27.

4. Thoulouze M-I, Bouguyon E, Carpentier C, Brémont M. Essential role of the NV protein of Novirhabdovirus for pathogenicity in rainbow trout. Journal of virology. 2004; 78(8):4098–107. https://doi.org/10.1128/jvi.78.8.4098-4107.2004 PMID: 15047826

5. Choi MK, Moon CH, Ko MS, Lee U-H, Cho WJ, Cha SJ, et al. A nuclear localization of the infectious haematopoietic necrosis virus NV protein is necessary for optimal viral growth. PLoS One. 2011; 6(7).

6. Bootland JCL L.M. Infectious haematopoietic necrosis virus. in: Woo DWBE P.T.K., editor. New York CABI Publishing; 1999. 57–121 p.

7. Rucker WJW R.R., Parvin J.R., Evans C.A. A contagious disease of sockeye salmon possibly of virus origin. US Fish Wildlife Service Fisheries Bulletin 1953; 54:35–46.

8. Wolf K. Viral hemorrhagic septicemia. Fish viruses and fish viral diseases. Cornell University Pres/Ithaca and Landon. 1988:217–49.

9. Dietzgen RG, Kuzmin IV. Rhabdoviruses: Molecular Taxonomy, Evolution, Genomics, Ecology, Host-vector Interactions, Cytopathology, and Control: Horizon Scientific Press; 2012.

10. Kimura T, Yoshimizu M. Viral diseases of fish in Japan. Annual Review of Fish Diseases. 1991; 1:67–82. https://doi.org/10.1016/0959-8030(91)90023-D PMID: 32288348

11. Park M, Sohn S, Lee S, Chun S, Park J, Fryer J, et al. Infectious haematopoietic necrosis virus from salmonids cultured in Korea. Journal of Fish Diseases. 1993; 16(5):471–8.

12. Jia P, Zheng X-C, Shi X-J, Kan S-F, Wang J-J, He J-Q, et al. Determination of the complete genome sequence of infectious hematopoietic necrosis virus (IHNV) Ch20101008 and viral molecular evolution in China. Infection, Genetics and Evolution. 2014; 27:418–31. https://doi.org/10.1016/j.meegid.2014.08.013 PMID: 25172153

13. Adel M, Amiri AB, Dadar M, Breyta R, Kurath G, Laktarashi B, et al. Phylogenetic relationships of Iranian infectious hematopoietic necrosis virus of rainbow trout (Oncorhynchus mykiss) based on the glycoprotein gene. Archives of virology. 2016; 161(3):657–63. https://doi.org/10.1007/s00705-015-2684-8 PMID: 26602428

14. Jensen MH. Research on the virus of Egtved disease. Annals of the New York Academy of Sciences. 1965; 126(1):422–6. https://doi.org/10.1111/j.1749-6632.1965.tb14292.x PMID: 4957057

15. Mortensen HF, Heuer OE, Lorenzen N, Otte L, Olesen NJ. Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild marine fish species in the Baltic Sea, Kattegat, Skagerrak and the North Sea. Virus research. 1999; 63(1–2):95–106. https://doi.org/10.1016/s0168-1702(99)00062-3 PMID: 10509720

16. Hedrick R, Batts W, Yun S, Traxler G, Kaufman J, Winton J. Host and geographic range extensions of the North American strain of viral hemorrhagic septicemia virus. Diseases of aquatic organisms. 2003; 55(3):211–20. https://doi.org/10.3354/dao055211 PMID: 13677507

17. Takano R, Nishizawa T, Arimoto M, Muroga K. Isolation of viral haemorrhagic septicaemia virus (VHSV) from wild Japanese flounder, Paralichthys olivaceus. Bulletin-European Association of Fish Pathologists. 2000; 20(5):186–92.

18. Isshiki T, Nishizawa T, Kobayashi T, Nagano T, Miyazaki T. An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed Japanese flounder Paralichthys olivaceus in Japan. Diseases of aquatic organisms. 2001; 47(2):87–99. https://doi.org/10.3354/dao047087 PMID: 11775799

19. Takano R, Mori K-i, Nishizawa T, Arimoto M, Muroga K. Isolation of viruses from wild Japanese flounder Paralichthys olivaceus. Fish Pathology. 2001; 36(3):153–60.

20. Kim S-M, Lee J-I, Hong M-J, Park H-S, Park S-I. Genetic relationship of the VHSV (viral hemorrhagic septicemia virus) isolated from cultured olive flounder, Paralichthys olivaceus in Korea. Journal of fish pathology. 2003; 16(1):1–12.

21. Kim W-S, Kim S-R, Kim D, Kim J-O, Park M-A, Kitamura S-I, et al. An outbreak of VHSV (viral hemorrhagic septicemia virus) infection in farmed olive flounder Paralichthys olivaceus in Korea. Aquaculture. 2009; 296(1–2):165–8.

22. Kimura T, Yoshimizu M, Gorie S. A new rhabdovirus isolated in Japan from cultured hirame (Japanese flounder) Paralichthys olivaceus and ayu Plecoglossus altivelis. Diseases of Aquatic Organisms. 1986; 1(3):209–17.

23. Oh M-J, Choi T-J. A new rhabdovirus (HRV-like) isolated in Korea from cultured Japanese flounder Paralichthys olivaceus. Journal of fish pathology. 1998; 11(2):129–36.

24. Yingjie S, Min Z, Hong L, Zhiqin Y, Xiaocong Z, Zhe W. Analysis and characterization of the complete genomic sequence of the Chinese strain of hirame rhabdovirus. Journal of fish diseases. 2011; 34(2):167–71. https://doi.org/10.1111/j.1365-2761.2010.01218.x PMID: 21241324

25. Borzym E, Matras M, Maj-Paluch J, Baud M, De Boisséson C, Talbi C, et al. First isolation of hirame rhabdovirus from freshwater fish in E urope. Journal of fish diseases. 2014; 37(5):423–30. https://doi.org/10.1111/jfd.12119 PMID: 23962315

26. Seo HG, Do J, Jung S, Han HJ. Outbreak of hirame rhabdovirus infection in cultured spotted sea bass Lateolabrax maculatus on the western coast of Korea. Journal of fish diseases. 2016; 39(10):1239–46. https://doi.org/10.1111/jfd.12513 PMID: 27371509

27. Webby R, Hoffmann E, Webster R. Molecular constraints to interspecies transmission of viral pathogens. Nature medicine. 2004; 10(12):S77–S81.

28. Moya A, Holmes EC, González-Candelas F. The population genetics and evolutionary epidemiology of RNA viruses. Nature Reviews Microbiology. 2004; 2(4):279–88. https://doi.org/10.1038/nrmicro863 PMID: 15031727

29. McNab F, Mayer-Barber K, Sher A, Wack A, O'garra A. Type I interferons in infectious disease. Nature Reviews Immunology. 2015; 15(2):87–103. https://doi.org/10.1038/nri3787 PMID: 25614319

30. Le CP, Genin P, Baines M, Hiscott J. Interferon activation and innate immunity. Reviews in immunogenetics. 2000; 2(3):374–86. PMID: 11256746

31. Haller O, Kochs G, Weber F. The interferon response circuit: induction and suppression by pathogenic viruses. Virology. 2006; 344(1):119–30. https://doi.org/10.1016/j.virol.2005.09.024 PMID: 16364743

32. Versteeg GA, García-Sastre A. Viral tricks to grid-lock the type I interferon system. Current opinion in microbiology. 2010; 13(4):508–16. https://doi.org/10.1016/j.mib.2010.05.009 PMID: 20538505

33. Purcell MK, Laing KJ, Winton JR. Immunity to fish rhabdoviruses. Viruses. 2012; 4(1):140–66. https://doi.org/10.3390/v4010140 PMID: 22355456

34. Randall RE, Goodbourn S. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. Journal of general virology. 2008; 89(1):1–47.

35. De Maeyer E, Enders JF. Growth characteristics, interferon production and plaque formation with different lines of Edmonston measles virus. Archiv für die gesamte Virusforschung. 1965; 16(1–5):151–60.

36. Ruiz-Gomez J, Isaacs A. Interferon production by different viruses. Virology. 1963; 19(1):8–12.

37. Sellers R. Multiplication, interferon production and sensitivity of virulent and attenuated strains of the virus of foot-and-mouth disease. Nature. 1963; 198(4886):1228–9.

38. Kasai H, Yoshimizu M. Establishment of two Japanese flounder embryo cell lines. Bull Fish Sci Hokkaido Univ. 2001; 52:67–70.

39. Winton J, Batts W, deKinkelin P, LeBerre M, Bremont M, Fijan N. Current lineages of the epithelioma papulosum cyprini (EPC) cell line are contaminated with fathead minnow, Pimephales promelas, cells. J Fish Dis. 2010; 33:701–4. https://doi.org/10.1111/j.1365-2761.2010.01165.x PMID: 20497291

40. Batts W, Winton J. Enhanced detection of infectious hematopoietic necrosis virus and other fish viruses by pretreatment of cell monolayers with polyethylene glycol. Journal of Aquatic Animal Health. 1989; 1(4):284–90.

41. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-ΔΔCt) method. Methods 2001; 25:402–8. https://doi.org/10.1006/meth.2001.1262 PMID: 11846609

42. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature protocols. 2012; 7 (3):562–78. https://doi.org/10.1038/nprot.2012.016 PMID: 22383036

43. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. Nucleic acids research. 2012; 41(D1):D808–D15.

44. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, et al. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nature protocols. 2013; 8(8):1494. https://doi.org/10.1038/nprot.2013.084 PMID: 23845962

45. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102(43):15545–50 https://doi.org/10.1073/pnas.0506580102 PMID: 16199517

46. Purcell MK, Kurath G, Garver KA, Herwig RP, Winton JR. Quantitative expression profiling of immune response genes in rainbow trout following infectious haematopoietic necrosis virus (IHNV) infection or DNA vaccination. Fish & shellfish immunology. 2004; 17(5):447–62.

47. Penaranda MMD, Purcell MK, Kurath G. Differential virulence mechanisms of infectious hematopoietic necrosis virus in rainbow trout (Oncorhynchus mykiss) include host entry and virus replication kinetics. Journal of General Virology. 2009; 90(9):2172–82.

48. Purcell MK, LaPatra SE, Woodson JC, Kurath G, Winton JR. Early viral replication and induced or constitutive immunity in rainbow trout families with differential resistance to Infectious hematopoietic necrosis virus (IHNV). Fish & shellfish immunology. 2010; 28(1):98–105.

49. Bernard J, Bearzotti-Le Berre M, De Kinkelin P, editors. Viral haemorrhagic septicaemia in rainbow trout: attempt to relate interferon production, antibody synthesis and structure of the virus with the mechanism of virulence. Annales de l'Institut Pasteur/Virologie; 1985: Elsevier.

50. Kinkelin PD, Dorson M, Hattenberger-Baudouy A. Interferon synthesis in trout and carp after viral infection. Developmental and comparative immunology. 1982;(Suppl. 2):167–74.

51. Lorenzen N, Lorenzen E, Einer-Jensen K, LaPatra SE. Immunity induced shortly after DNA vaccination of rainbow trout against rhabdoviruses protects against heterologous virus but not against bacterial pathogens. Developmental & Comparative Immunology. 2002; 26(2):173–9.

52. LaPatra SE, Corbeil S, Jones GR, Shewmaker WD, Lorenzen N, Anderson ED, et al. Protection of rainbow trout against infectious hematopoietic necrosis virus four days after specific or semi-specific DNA vaccination. Vaccine. 2001; 19(28–29):4011–9. https://doi.org/10.1016/s0264-410x(01)00113-x PMID: 11427277

53. Verjan ELO N., Nochi T., Kondo H., Hirono I., Aoki T., Kiyono H., et al A soluble nonglycosylated recombinant infectous hematopoietic necrosis virus (IHNV) G-protein induces IFNs in rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 2008; 25:170–80. https://doi.org/10.1016/j.fsi.2008.04.004 PMID: 18499475

54. Nelson N, Marks MS, Driggers PH, Ozato K. Interferon consensus sequence-binding protein, a member of the interferon regulatory factor family, suppresses interferon-induced gene transcription. Molecular and cellular biology. 1993; 13(1):588–99. https://doi.org/10.1128/mcb.13.1.588 PMID: 7678054

55. White CL, Kessler PM, Dickerman BK, Ozato K, Sen GC. Interferon regulatory factor 8 (IRF8) impairs induction of interferon induced with tetratricopeptide repeat motif (IFIT) gene family members. Journal of Biological Chemistry. 2016; 291(26):13535–45. https://doi.org/10.1074/jbc.M115.705467 PMID: 27137933

56. Huang W, Horvath E, Eklund EA. PU. 1, interferon regulatory factor (IRF) 2, and the interferon consensus sequence-binding protein (ICSBP/IRF8) cooperate to activate NF1 transcription in differentiating myeloid cells. Journal of Biological Chemistry. 2007; 282(9):6629–43. https://doi.org/10.1074/jbc.M607760200 PMID: 17200120

57. Li P, Wong JJ-Y, Sum C, Sin W-X, Ng K-Q, Koh MB, et al. IRF8 and IRF3 cooperatively regulate rapid interferon-β induction in human blood monocytes. Blood, The Journal of the American Society of Hematology. 2011; 117(10):2847–54.

58. Tsujimura H, Tamura T, Gongora C, Aliberti J, Reis e Sousa C, Sher A, et al. ICSBP/IRF-8 retrovirus transduction rescues dendritic cell development in vitro. Blood, The Journal of the American Society of Hematology. 2003; 101(3):961–9.

59. Sun L, Leger AJS, Yu C-R, He C, Mahdi RM, Chan C-C, et al. Interferon regulator factor 8 (IRF8) limits ocular pathology during HSV-1 infection by restraining the activation and expansion of CD8+ T Cells. PLoS One. 2016; 11(5).

60. Adams NM, Lau CM, Fan X, Rapp M, Geary CD, Weizman O-E, et al. Transcription factor IRF8 orchestrates the adaptive natural killer cell response. Immunity. 2018; 48(6):1172–82. e6. https://doi.org/10.1016/j.immuni.2018.04.018 PMID: 29858012

61. Hu G, Chen X, Gong Q, Liu Q, Zhang S, Dong X. Structural and expression studies of interferon regulatory factor 8 in Japanese flounder, Paralichthys olivaceus. Fish & shellfish immunology. 2013; 35(3):1016–24.

62. Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. Cytokine & growth factor reviews. 1998; 9(1):9–23.

63. Bird S, Tafalla C. Teleost chemokines and their receptors. Biology. 2015; 4(4):756–84. https://doi.org/10.3390/biology4040756 PMID: 26569324

64. Tafalla C, Coll J, Secombes C. Expression of genes related to the early immune response in rainbow trout (Oncorhynchus mykiss) after viral haemorrhagic septicemia virus (VHSV) infection. Developmental & Comparative Immunology. 2005; 29(7):615–26.

65. Jimenez N, Coll J, Salguero F, Tafalla C. Co-injection of interleukin 8 with the glycoprotein gene from viral haemorrhagic septicemia virus (VHSV) modulates the cytokine response in rainbow trout (Oncorhynchus mykiss). Vaccine. 2006; 24(27–28):5615–26. https://doi.org/10.1016/j.vaccine.2006.04.061 PMID: 16725233

66. Khabar KS, Al-Zoghaibi F, Murayama T, Matsushima K, Mukaida N, Siddiqui Y, et al. Interleukin-8 selectively enhances cytopathic effect (CPE) induced by positive-strand RNA viruses in the human WISH cell line. Biochemical and biophysical research communications. 1997; 235(3):774–8. https://doi.org/10.1006/bbrc.1997.6872 PMID: 9207237

67. Murayama T, Kuno K, Jisaki F, Obuchi M, Sakamuro D, Furukawa T, et al. Enhancement human cytomegalovirus replication in a human lung fibroblast cell line by interleukin-8. Journal of virology. 1994; 68(11):7582–5. https://doi.org/10.1128/JVI.68.11.7582-7585.1994 PMID: 7933146

68. Horng T, Barton GM, Medzhitov R. TIRAP: an adapter molecule in the Toll signaling pathway. Nature immunology. 2001; 2(9):835–41. https://doi.org/10.1038/ni0901-835 PMID: 11526399

69. Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, et al. Mal (MyD88-adapter-like) is required for Toll-like receptor-4 signal transduction. Nature. 2001; 413 (6851):78–83. https://doi.org/10.1038/35092578 PMID: 11544529

70. Bonham KS, Orzalli MH, Hayashi K, Wolf AI, Glanemann C, Weninger W, et al. A promiscuous lipid-binding protein diversifies the subcellular sites of toll-like receptor signal transduction. Cell. 2014; 156 (4):705–16. https://doi.org/10.1016/j.cell.2014.01.019 PMID: 24529375

71. Ahmad R, El Bassam S, Cordeiro P, Menezes J. Requirement of TLR2-mediated signaling for the induction of IL-15 gene expression in human monocytic cells by HSV-1. Blood, The Journal of the American Society of Hematology. 2008; 112(6):2360–8.

72. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15–deficient mice. The Journal of experimental medicine. 2000; 191(5):771–80. https://doi.org/10.1084/jem.191.5.771 PMID: 10704459

73. Patel A, Hanson J, McLean TI, Olgiate J, Hilton M, Miller WE, et al. Herpes simplex virus type 1 induction of persistent NF-κB nuclear translocation increases the efficiency of virus replication. Virology. 1998; 247(2):212–22. https://doi.org/10.1006/viro.1998.9243 PMID: 9705914

74. Park ALS K. Cholesterol 25-hydroxylase production by dendritic cells and macrophages is regulated by type I interferons. J Leukoc Biol. 2010; 88:1081–7. https://doi.org/10.1189/jlb.0610318 PMID: 20699362

75. Liu S-Y, Aliyari R, Chikere K, Li G, Marsden MD, Smith JK, et al. Interferon-inducible cholesterol-25-hydroxylase broadly inhibits viral entry by production of 25-hydroxycholesterol. Immunity. 2013; 38 (1):92–105. https://doi.org/10.1016/j.immuni.2012.11.005 PMID: 23273844

76. Blanc M, Hsieh WY, Robertson KA, Kropp KA, Forster T, Shui G, et al. The transcription factor STAT-1 couples macrophage synthesis of 25-hydroxycholesterol to the interferon antiviral response. Immunity. 2013; 38(1):106–18. https://doi.org/10.1016/j.immuni.2012.11.004 PMID: 23273843

77. Chen Y, Wang S, Yi Z, Tian H, Aliyari R, Li Y, et al. Interferon-inducible cholesterol-25-hydroxylase inhibits hepatitis C virus replication via distinct mechanisms. Scientific reports. 2014; 4(1):1–8.

78. Pereiro P, Forn-Cuni G, Dios S, Coll J, Figueras A, Novoa B. Interferon-independent antiviral activity of 25-hydroxycholesterol in a teleost fish. Antiviral Res 2017; 145:146–59. https://doi.org/10.1016/j.antiviral.2017.08.003 PMID: 28789986

79. Zhang Y, Wang L, Huang X, Wang S, Huang Y, Qin Q. Fish cholesterol 25-hydroxylase inhibits virus replication via regulating interferon immune response or affecting virus entry. Frontiers in Immunology 2019; 10:322. https://doi.org/10.3389/fimmu.2019.00322 PMID: 30894855

80. de Oliviera Nascimento L, Massari P, Wetzler LM. The role of TLR2 in infection and immunity. Frontiers in immunology. 2012; 3:79. https://doi.org/10.3389/fimmu.2012.00079 PMID: 22566960

81. Zähringer U, Lindner B, Inamura S, Heine H, Alexander C. TLR2–promiscuous or specific? A critical re-evaluation of a receptor expressing apparent broad specificity. Immunobiology. 2008; 213(3–4):205–24. https://doi.org/10.1016/j.imbio.2008.02.005 PMID: 18406368

82. Xagorari A, Chlichlia K. Toll-like receptors and viruses: induction of innate antiviral immune responses. The open microbiology journal. 2008; 2:49. https://doi.org/10.2174/1874285800802010049 PMID: 19088911

83. Barbalat R, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. Nature immunology. 2009; 10(11):1200. https://doi.org/10.1038/ni.1792 PMID: 19801985

84. Szomolanyi-Tsuda E, Liang X, Welsh RM, Kurt-Jones EA, Finberg RW. Role for TLR2 in NK cell-mediated control of murine cytomegalovirus in vivo. Journal of virology. 2006; 80(9):4286–91. https://doi.org/10.1128/JVI.80.9.4286-4291.2006 PMID: 16611887

85. Avunje S, Oh M-J, Jung S-J. Impaired TLR2 and TLR7 response in olive flounder infected with viral haemorrhagic septicaemia virus at host susceptible 15˚C but high at non-susceptible 20˚C. Fish & shellfish immunology. 2013; 34(5):1236–43.

86. Pereiro P, Dios S, Boltana S, Coll J, Estepa A, Mackenzie S, et al. Transcriptome profiles associated to VHSV infection or DNA vaccination in turbot (Scophthalmus maximus). PloS One. 2014; 9(8).

87. Liu Y, Chang M, Wu S, Nie P. Characterization of C–C chemokine receptor subfamily in teleost fish. Molecular immunology. 2009; 46(3):498–504. https://doi.org/10.1016/j.molimm.2008.10.003 PMID: 19022503

88. Wang L, Zhang Y-z, Xu W-t, Jia X-d, Chen S-l. Molecular cloning, structure and expressional profiles of two novel single-exon genes (PoCCR6A and PoCCR6B) in the Japanese flounder (Paralichthys olivaceus). Fish & shellfish immunology. 2016; 52:179–88.

89. Schutyser E, Struyf S, Van Damme J. The CC chemokine CCL20 and its receptor CCR6. Cytokine & growth factor reviews. 2003; 14(5):409–26.

90. Punj V, Matta H, Schamus S, Yang T, Chang Y, Chaudhary PM. Induction of CCL20 production by Kaposi sarcoma–associated herpesvirus: role of viral FLICE inhibitory protein K13-induced NF-κB activation. Blood, The Journal of the American Society of Hematology. 2009; 113(22):5660–8.

91. Kallal LE, Schaller MA, Lindell DM, Lira SA, Lukacs NW. CCL20/CCR6 blockade enhances immunity to RSV by impairing recruitment of DC. European journal of immunology. 2010; 40(4):1042–52. https://doi.org/10.1002/eji.200939778 PMID: 20101616

92. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annual review of immunology. 1995; 13 (1):251–76.

93. Rigopoulou EI, Suri D, Chokshi S, Mullerova I, Rice S, Tedder RS, et al. Lamivudine plus interleukin-12 combination therapy in chronic hepatitis B: antiviral and immunological activity. Hepatology. 2005; 42(5):1028–36. https://doi.org/10.1002/hep.20888 PMID: 16250037

94. Rossol S, Marinos G, Carucci P, Singer MV, Williams R, Naoumov NV. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. The Journal of clinical investigation. 1997; 99(12):3025–33. https://doi.org/10.1172/JCI119498 PMID: 9185527

95. Tsuge M, Takahashi S, Hiraga N, Fujimoto Y, Zhang Y, Mitsui F, et al. Effects of hepatitis B virus infection on the interferon response in immunodeficient human hepatocyte chimeric mice. Journal of Infectious Diseases. 2011; 204(2):224–8. https://doi.org/10.1093/infdis/jir247 PMID: 21673032

96. Boehme KW, Guerrero M, Compton T. Human cytomegalovirus envelope glycoproteins B and H are necessary for TLR2 activation in permissive cells. The Journal of Immunology. 2006; 177(10):7094–102. https://doi.org/10.4049/jimmunol.177.10.7094 PMID: 17082626

97. Miettinen M, Sareneva T, Julkunen I, Matikainen S. IFNs activate toll-like receptor gene expression in viral infections. Genes & Immunity. 2001; 2(6):349–55.

98. Hirono I, Takami M, Miyata M, Miyazaki T, Han H-J, Takano T, et al. Characterization of gene structure and expression of two toll-like receptors from Japanese flounder, Paralichthys olivaceus. Immunogenetics. 2004; 56(1):38–46. https://doi.org/10.1007/s00251-004-0657-2 PMID: 15007570

99. Cho S-Y, Protzman RA, Kim YO, Vaidya B, Oh M-J, Kwon J, et al. Elucidation of mechanism for host response to VHSV infection at varying temperatures in vitro and in vivo through proteomic analysis. Fish & Shellfish Immunology. 2019; 88:244–53.

100. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nature Immunology 2015; 16: 343–53 https://doi.org/10.1038/ni.3123 PMID: 25789684

101. Hwang JY, Kwon MG, Jung S-H, Park MA, Kim D-W, Cho WS, et al. RNA-Seq transcriptome analysis of the olive flounder (Paralichthys olivaceus) kidney response to vaccination with heat-inactivated viral hemorrhagic septicemia virus. Fish & Shellfish Immunology 2017; 62:221–6

102. Hwang JY, Markkandan K, Kwon MG, Seo JS, Yoo S-I, Hwang SD, et al. Transcriptome analysis of olive flounder (Paralichthys olivaceus) head kidney infected with moderate and high virulent strains of infectious viral hemorrhagic septicaemia virus (VHSV). Fish & Shellfish Immunology 2018; 76:293–304

103. Hwang JY, Markkandan K, Han K, Kwon MG, Seo JS, Yoo S-I, et al. Temperature-dependent immune response of olive flounder (Paralichthys olivaceus) infected with viral hemorrhagic septicemia virus (VHSV). Genes Genomics 2018; 40:315–20. https://doi.org/10.1007/s13258-017-0638-0 PMID: 29892801

104. Byon JY, Ohira T, Hirono I, Aoki TJF. Use of a cDNA microarray to study immunity against viral hemorrhagic septicemia (VHS) in Japanese flounder (Paralichthys olivaceus) following DNA vaccination. Fish & Shellfish Immunology 2005; 18:135–47

105. Byon JY, Ohira T, Hirono I, Aoki TJV. Comparative immune responses in Japanese flounder, Paralichthys olivaceus after vaccination with viral hemorrhagic septicemia virus (VHSV) recombinant glycoprotein and DNA vaccine using a microarray analysis. Vaccine 2006; 24:921–30 https://doi.org/10.1016/j.vaccine.2005.08.087 PMID: 16185791

106. Hwang JY, Kwon M-G, Seo JS, Do JW, Park M-A, Jung S-H, et al. Differentially expressed genes after viral haemorrhagic septicaemia virus infection in olive flounder (Paralichthys olivaceus). Veterinary Microbiology 2016; 193:72–82 https://doi.org/10.1016/j.vetmic.2016.05.024 PMID: 27599933

107. Cho HK, Kim J, Moon JY, Nam BH, Kim YO, Kim WJ, et al. Microarray analysis of gene expression in olive flounder liver infected with viral haemorrhagic septicaemia virus (VHSV). Fish & Shellfish Immunology 2016; 49:66–78

108. Purcell MK, Marjara IS, Batts W, Kurath G. Transcriptome analysis of rainbow trout infected with high and low virulence strains of Infectious hematopoietic necrosis virus. Fish & Shellfish Immunology 2010; 30:84–93

109. Polinski MP, Bradshaw JC, Rise ML, Johnson SC, Garver KA. Sockeye salmon demonstrate robust yet distinct transcriptomic kidney responses to rhabdovirus (IHNV) exposure and infection. Fish & Shellfish Immunology 2019; 94:525–38