The Israeli Journal of Aquaculture – Bamidgeh • ISSN 0792-156X • IJA.74.2022.1774523, 10 pages
CCBY-NC-ND-4.0 ● https://doi.org/10.46989/001c.38010

Comparative analysis of differential gene expression in two species of crucian carps in response to Cyprinid herpesvirus 2 (CyHV-2) infection

Yanming Sui[1], Jiyi Chen[1], Weiwei Ji[2], Wen Ruan[2], Yayan Xu[2], Liang Zheng[2]*, Yanan Lu[2]*

[1] *College of Marine and Biological Engineering, Yancheng Institute of Technology, Yancheng, China*

[2] *East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China*

Keywords: Carp; Cyprinid herpesvirus 2; Gene expression

Abstract

We assessed the expressions of *MHC*I, *LYZC*, *keratin8*, *MPO*, *DUSP1*, *IκBα*, *Rab21,* and *Rac2* between two species of carps (Erqisi river crucian carp and allogynogenetic crucian carp) after Cyprinid herpesvirus 2 (CyHV-2) infection. The relative expressions of *MHC*I, *LYZC,* and k*eratin8* in the virus-challenged groups were significantly higher than control groups. Moreover, the expression of *IκBα* in the virus-challenged groups was significantly lower than in the control groups. Compared with the virus-challenged ERO group, the expression of *IκBα* in the virus-challenged ZHO group decreased. The expression of *Rab21* in the virus-challenged groups gradually increased and was significantly higher than in the control groups, and then its expression began to decrease after 24 h. At 72 h, the expression of *IκBα* in both virus-challenged groups was significantly lower than in the control groups. In addition, the expression of *Rab21* in the virus-challenged ZHO group was significantly higher than the virus-challenged ERO group at all time points except for 72 h. Before 24 h, the expression of *Rac2* remained unchanged in these four groups, and its expression in the virus-challenged ZHO group was significantly higher than in the other three groups. Nevertheless, its expression began to decrease after 24 h but was still slightly higher than the control group at 72 h. *MPO* showed a similar expression pattern as *Rac2*. The expression of *DUSP1* in the four groups was the same at 0 h. However, its expression in the virus-challenged ZHO group was significantly higher than in the other three groups at other time points.

* Corresponding author. * Liang Zheng e-mail: kingfishercheng@163.com * Yannan Lu e-mail: luyn@ecsf.ac.cn

Introduction

Erqisi river crucian carp (*Carassius auratus gibelio*) and allogynogenetic crucian carp (*Carassius auratus gibelio*) are important freshwater fish species in China. Erqisi river crucian carp is mainly distributed in Xinjiang Uygur Autonomous Region, which is a fast-growth specie with a favorable taste. Allogynogenetic crucian carp is a widely cultured fish species in China with over one million tons of production every year. However, they are susceptible to some viral infections. For instance, when fish are infected with Cyprinid herpesvirus 2 (CyHV-2), they will die within 2 days after some clinical signs appear (Xu et al., 2014).

CyHV-2 is one of the most serious viruses in crucian carp, and it is the second herpesvirus we found in Cyprinid. This virus is widely distributed all over the world, including Japan, America and China, and it is reported firstly in Japan (Jung and Miyazaki, 2010). Moreover, it can cause death of carp, such as the high mortality of goldfish in Taiwan (Chang et al., 1999). This virus always appears in summer and autumn, especially when the water temperature is between 15 to 25 °C (Kong et al., 2017). There are some similar symptoms in infected fish, such as bleeding, pale gills, ascites, abnormal spleen and kidneys (Podok et al., 2014).

It is convinced that *MHC*I exists in each vertebrate (Bensaid et al., 1991), and the molecular structure of *MHC*I in fish is similar to that in mammals. *MHC*I is found firstly in the carp (Hashimoto et al., 1990), and it has been detected in several other fish pieces (Xu et al., 2011; Pinto et al., 2013). As the critical immune gene of fish, it plays an important role in the immune response regulation and antigen processing. Lysozyme is a non-specific immune protein factor that cures the disease directly by hydrolyzing the peptides in the cell walls of bacteria. Lysozyme can be divided into six types, and LYZC is one of them. LYZC accounts for the largest proportion in nature and exists in both vertebrates and invertebrates, and it is firstly reported in *Oncorhynchus mykiss* (Dautigny et al., 1991). It is an important non-specific immune factor that can protect fish from pathogens. *Keratin8* exists mainly in gastrointestinal tract and liver, and it plays an important role in inflammatory response, cell growth and prophylaxis of tumor (Majumdar et al., 2012). *MPO* is expressed in cytoplasmic granules of myeloid cells, and it is critical in the microbial infections (Kettle et al., 1993). *DUSP1* is the negative regulatory gene of MAPK family protein, and it participates in a large number of biological processes, including cell signaling, chondrocyte growth and cell metabolism. Recently, it has been found that *DUSP1* can inhibit the growth of hepatocellular carcinoma cells by regulating EKR (Calvisi et al., 2008). In eukaryotic cells, *IκBα/NF-Kb* is widespread, which can be enhanced with a variety of cellular genes or sequences. *IκBα/NF-Kb* can promote the transcription and expression of related genes, and they are closely related to the important pathophysiological processes, such as stress response, immune regulation, inflammation, growth control, embryonic development, cell hyperplasia, transformation and apoptosis (Chakraborty and Mann, 2010). *Rab21* is involved in the transportation of endocytic vesicles from early endocytic compartment to late endocytic compartment. Moreover, it can interact with integrin within the cell membrane, mediating cell movement and adhesion (Pellinen et al., 2006; Simpson et al., 2004). *Rac2* is an important regulatory factor in the cell signal transduction and formation of actin cytoskeleton. It mainly exists at phagosome membrane and can participate in the formation of hematopoietic cells as well as regulation of cell signal transduction and actin cytoskeleton (Pradip et al., 2003; De et al., 2009).

In the present study, we aimed to examine the expressions of some innate immune-related genes (*MHC*I, *LYZC*, *keratin8*, *MPO*, *DUSP1*, *IκBa*, *Rab21* and *Rac2*) between two species, and previous studies have confirmed that these genes are differentially expressed in crucian carp in response to viral infection (Xu et al., 2016). The purpose is to determine which species has the superiority on anti-virus and help to accumulate fundamental data in crucian carp breeding.

Materials and Methods

Experimental animal

The fishes used in this study were Erqisi river crucian carp and allogynogenetic crucian carp with the similar specifications, which were assigned as ERO and ZHO, respectively. The average body weight of the fishes used in the experiment were (200±10) g. Erqisi river crucian carp were cultured in Erqisi River, Xinjiang Uygur Autonomous Region (133 thousand individuals/mu in farming density), and allogynogenetic crucian carp were fed in Dafeng City, Jiangsu Province (444 thousand individuals/mu in farming density).

For the infection group, fish were injected with 1 mL of 10^6 viral particles mL of CyHV-2 in the abdominal cavity according to the previous method (Xia et al., 2016). Fishes were injected with 1 mL of PBS as the control group. All fishes were maintained in water at a controlled temperature between 20 to 23 °C which was suitable for their living.

Sample collection

After VLPs infection, the expressions of immune-related genes were examined within 72 h. A total of 18 fishes from the infection group were collected for each species, and the same number of fishes were collected from the control group. The kidney tissue was collected at 0, 6, 12, 24, 48 and 72 h post-injection.

PCR was used to confirm viral infection. In each group, two extra replicates were gathered, and all samples were refrigerated at -20° C prior to real-time PCR.

RNA extraction and cDNA synthesis

Total RNA was extracted from kidney tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The kidney tissue for each individual was set as one sample. The purity of the isolated RNA was determined based on the $OD_{260\ nm}/OD_{280\ nm}$ ratio, with expected values between 1.8 and 2.0. RNase free-DNase I (Takara, Japan) was used to remove residual genomic DNA, and then the purified RNA was reversely transcribed into cDNA using random hexamer primers and MMLV Reverse Transcriptase (Takara) according to the manufacturer's instructions.

Primer design and source

For designing primers for amplification of the eight genes, different set of *MHC*I, *LYZC*, *keratin8*, *MPO*, *DUSP1*, *IκBα*, *Rab21* and *Rac2* sequences of heterologous fish species such as *Cyprinus carpio* (AB018581.1), *Carassius auratus* (KJ703112.1), Ctenopharyngodon Idella (KY081642.1), *Cyprinus carpio* (HE584636.1), *Danio rerio* (AF201451.1 and AY057094.1) and *Megalobrama amblycephala* (JQ905614.1) were obtained from NCBI database. The sequences of primers are listed as follow:

*MHC*I: Fw: CTCATCTCCAGTCGTGTGTCA/Rv: AAAGGTCCCGTCATCATTAGG
LYZC: Fw: ACTTGATGGCTTTGAGGGATT/Rv: TTACACTGGTTCTTTCCACCTG
keratin8: Fw: GTTGAGAGGGAAGGTCAGGAAT/Rv: CAAGGATGGCAGAGTTGTGTC
MPO: Fw: CGGTCACTCTCTATGTCAGCA/Rv: GTATCTCCCAGCCCAAAGGT
DUSP1: Fw: TCTTCACTTCTCCCATCTCCA/Rv: CATTTTACCCAACGAGGACAC
IκBα: Fw: AATCTCTGTGCCAAACACTGG/Rv: GGGTGACTCCTCCTCATCG
Rab21: Fw: GAAATACCCCGTGAAGTTAGGA/Rv: GCAGTTGAATCTTTGTCTCCAG
Rac2: Fw: CAGAAGATTACGATGCCGATG/Rv: GTCCAGCCAGAGGGTTGTT

Real-time RT-PCR analysis

Real-time PCR was performed in a 20 μL reaction system consisting of 1 μL cDNA, 7 μL nuclease-free water, 10 μL 2× SsoAdvanced™ SYBR Green Supermix (Bio-Rad) and 1 μL of each specific primer (10 μM) on a CFX96™ Real-time PCR Detection System (Bio-Rad). β-actin was selected as the housekeeping gene. The relative expression levels of target genes were determined using the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen, 2012). Single-factor analysis of variance (*t*-test) was used to compare the differences between groups. *P* less than 0.05 was considered as statistically significant.

Results

Variations in the expression of eight genes in two species of crucian carp

In the present study, we examined the expressions of eight immune-related genes, including *MHC*I, *LYZC*, *keratin8*, *MPO*, *DUSP1*, *IκBα*, *Rab21* and *Rac2*, by real-time RT-PCR. From investigation, we can see the expression of *Rac2* (a) remained unchanged in these four groups, while its expression in the virus-challenged ZHO group was significantly higher compared the other three groups ($P < 0.05$) before 24 h. Nevertheless, the expression of *Rac2* was decreased at 72 h compared with other time points, but it was still slightly higher compared with the control group. The expression pattern of *MPO* (b) was similar to that of *Rac2*, except that its expression in the virus-challenged ZHO group was the highest at 48 h. The relative expression levels of *keratin8* (c) in the virus-challenged groups were significantly higher compared with the control groups ($P < 0.05$), and its expression in the virus-challenged ZHO group was significantly higher than that in the virus-challenged ERO group especially at 12 h ($P < 0.05$). The expression of *DUSP1*(d) in the four groups was the same at 0 h. However, its expression in the virus-challenged ZHO group was significantly higher compared the other three groups at other time points ($P < 0.05$, **Figure 1**).

As results show, the relative expression levels of *MHC*I (e) and *LYZC* (f) in the virus-challenged groups were significantly higher compared with the control groups ($P < 0.05$). Besides, there was a slight difference between the two virus-challenged groups. However, the expression of $I_{\kappa}B_{\alpha}$ (g) in the CyHV-2-challenged groups was significantly lower compared with the control groups ($P < 0.05$). Moreover, the expression of $I_{\kappa}B_{\alpha}$ in virus-challenged ZHO group was lower compared with the virus-challenged ERO group. From 0 h to 24 h, the expression of *Rab21* (h) in the virus-challenged groups gradually increased, which was significantly higher compared with the control groups ($P < 0.05$). However, its expression in the virus-challenged groups began to decrease after 24 h, which was significantly lower compared with the control groups at 72 h ($P < 0.05$). Moreover, the expression of *Rab21* in the virus-challenged ZHO group was significantly higher compared with the virus-challenged ERO group at all time points ($P < 0.05$) except for 72 h (**Figure 1**).

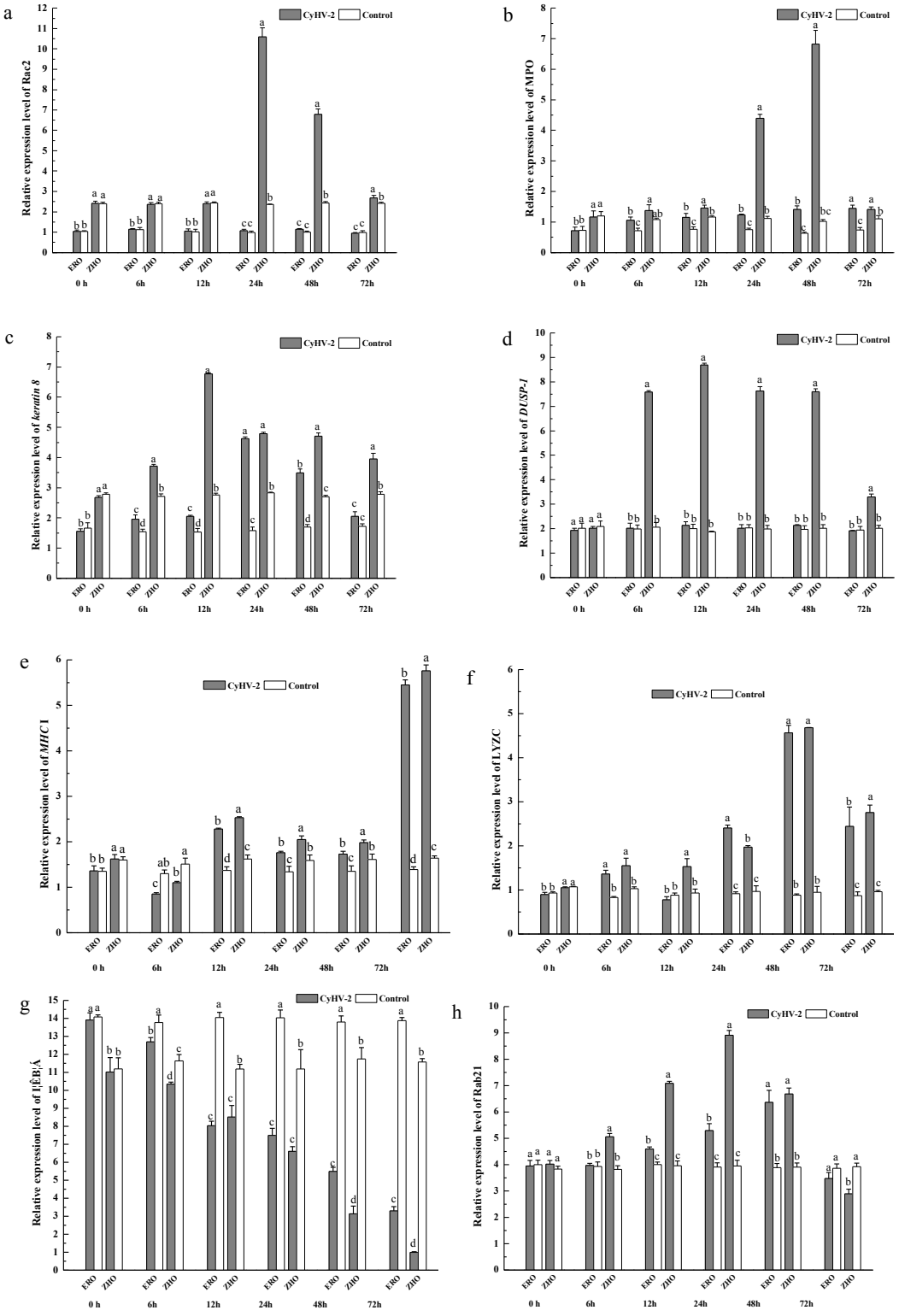

Figure 1 Relative expression levels of *Rac2*, *MPO*, *keratin8*, *DUSP1, MHC*I, *LYZC*, *IκBα* and *Rab21* in ERO and ZHO from 0 h to 72 h.
Note: different letters denote significant differences ($P<0.05$)

Discussion

In the present study, we assessed the expressions of eight immune-related genes in two different species of crucian carp through artificial infection of CyHV-2.

During 72-h infection, the expression of *MHC*I in both species of fish was significantly up-regulated, which was consistent with previous reports (Luo et al., 2014; Sever et al., 2014). Moreover, *MHC*I exists in each vertebrate (Bensaid et al., 1991), and it is required to deliver the antigen in the initial specific cellular immunity. We found that the expression of *MHC*I in the virus-challenged ZHO group was significantly higher compared with the virus-challenged ERO group, and its expression in both virus-challenged groups was significantly up-regulated compared with their own control group, reflecting a better initial specific cellular immunity in ZHO.

It has been confirmed that the expression of *LYZC* is high in many fish (Jiménezcantizano et al., 2008; Ye et al., 2010; Fernández-Trujillo et al., 2008), and its expression is up-regulated after bacterial infection (Minagawa et al., 2001; Wang et al., 2013). In our current study, we detected a similar expression pattern of *LYZC* compared with previous reports. Before 48 h, the expression of *LYZC* in the two virus-challenged groups was up-regulated, and then its expression began to decrease. In a previous report, the author found that the expression of *LYZC* in grass carp was up-regulated, and then its expression began to decrease, it was the same with ours (Ye et al., 2010).

The expression of *keratin8* in both virus-challenged groups was significantly up-regulated, and this finding was consistent with some previous reports (Ku et al., 2007; Podok et al., 2014). This up-regulation could be attributed that keratin protects the cell by falling off from host cells during the late stage in the apoptotic process (Schutte et al., 2004). Moreover, keratin is involved in liver disease by modulating disease progression upon mutation, and its expression is associated with chronic hepatitis C virus (Strnad et al., 2006). Besides, in previous experiments, *keratin8* plays a crucial role in protecting hepatocytes when mice are challenged by mechanical and toxic stresses (Fortier et al., 2010).

We found that the expression of *DUSP1* was different after 72h post-injection. Some previous studies have reported a similar expression pattern of *DUSP1* after infection with the vaccinia virus (Cáceres et al., 2013). The levels of pro-inflammatory and anti-inflammatory cytokines depend on *DUSP1* (Dickinson and Keyse, 2006). When fish undergo some stimulations, the expression of *DUSP-1* is significantly up-regulated (Lee et al., 2005; Liu et al., 2008; Shields et al., 2011). Similarly, the expression of *Rac2* in the virus-challenged ZHO group was significantly up-regulated. The expression of *Rac2* reflects the ability of this gene to trigger an inflammatory response, and *Rac2* plays an important role in the formation of reactive oxygen and nitrogen types (RONS)-dependent web-like structures (NET) in mice (Lim et al., 2011). However, its expression in the virus-challenged ERO group remained unchanged compared with the control group. The different expression patterns of *Rac2* between the two virus-challenged groups might be attributed to the amount of virus injection, which needs to be verified in the following experiment using gradient virus injection.

After CyHV-2 challenge, *IκBα* was the only down-regulated gene in two virus-challenged groups. In a previous study, when mandarin fish are infected with the spleen and kidney necrosis virus, the expression of *IκBα* is decreased (Wang et al., 2009). However, in other report, *IκBα* is up-regulated after being challenged with spring viremia of carp virus (Levraud et al., 2007). Such discrepancy could be explained by different infections (acute or chronic state).

The expression of *Rab21* in the virus-challenged ZHO group was significantly higher compared with the virus-challenged ERO group. *Rab21* is widely expressed and distributed (Ali et al., 2014), and it is involved in the transportation of endocytic vesicles. The expression of Rab21 affects the endo/exocytic transportation of interns (Pellinen et al., 2006). Moreover, its over-expression can suppress EGF-mediated mitogen-activated protein kinase signaling pathway, suggesting that *Rab21* plays a negative role in this signaling pathway (Xi et al.,

2012). According to above-mentioned findings, we believed that the difference in *Rab21* up-regulation between the two species of crucian carp could be attributed to that ZHO had a stronger ability to regulate the early endocytic pathway. After 72 h, there was no difference in the *Rab21* expression between the two species.

We found that the expression pattern of *MPO* was similar to that of *Rab21*. Previous studies have shown that the expression of *MPO* is significantly increased after CyHV-2 infection (Lau et al., 2005; Phung et al., 2012), which is consistent with our findings. Following trauma in zebrafish, *MPO*- and peroxidase-expressing cells are localized at the site of acute inflammation within several hours, leading to inflammation at the tip of the embryo's tail (Lieschke et al., 2001), and the expression of *MPO* is decreased after phagocytosis of bacteria (Bradley et al., 1982). These data are also consistent with our findings. The expression of *MPO* in both two species of challenged carp was significantly decreased at 72 h. The difference in its expression between two species of challenged carp at 24 h and 48 h might be attributed to the difference in anti-inflammatory path.

Acknowledgments

This work was supported by a grant from Central Public-interest Scientific Institution Basal Research Fund (No.2014T12).

References

Ali, M., Leung, K. F., Field. M. C., 2014. The ancient small GTPase rab21 functions in intermediate endocytic steps in trypanosomes. *Eukaryotic Cell*, 13(2), 304–319.
https://doi.org/10.1128/ec.00269-13

Bensaid, A., Kaushal, A., Baldwin, C. L., Clevers, H., Young, J. R., Kemp, S. J., Machugh, N. D., Toye, P. G., Teale, A. J., 1991. Identification of expressed bovine class I MHC genes at two loci and demonstration of physical linkage. *Immunogenetics*, 33, 247-254.
https://doi.org/10.1007/BF00230502

Bradley, P. P., Christensen, R. D., Rothstein, G., 1982. Cellular and extracellular myeloperoxidase in pyogenic inflammation. *Blood*, 60 (3), 618-622.
https://doi.org/10.0000/PMID6286012

Cáceres, A., Perdiguero, B., Gómez, C. E., Cepeda, M. V., Caelles, C., Sorzano, C. O., Esteban, M., 2013. Involvement of the cellular phosphatase DUSP1 in vaccinia virus infection. *PLoS Pathogens*, 9, e1003719.
https://doi.org/10.1371/journal.ppat.1003719

Calvisi, D. F., Pinna, F., Meloni, F., Ladu, S., Pellegrino, R., Sini, M., Daino, L., Simile, M. M., De Miglio, M. R., Virdis, P., 2008. Dual-specificity phosphatase 1 ubiquitination in extracellular signal-regulated kinase-mediated control of growth in human hepatocellular carcinoma. *Cancer Research*, 68, 4192-4200.
https://doi.org/10.1158/0008-5472.CAN-07-6157

Chakraborty, J. B., Mann, D. A., 2010. NF-kappaB signalling: embracing complexity to achieve translation. *Journal of Hepatology*, 52, 285-291.
https://doi.org/10.1016/j.jhep.2009.10.030

Chang, P. H., Shu, H. L., Chiang, H. C., Ming, H. J., 1999. Epizootic of herpes-like virus infection in *goldfish*, *Carassius auratus* in Taiwan. *Fish Pathology*, 34, 209-210.
https://doi.org/10.3147/jsfp.34.209

Dautigny, A., Prager, E. M., Pham-Dinh, D., Jollès, J., Pakdel, F., Grinde, B., Jollès, P., 1991. cDNA and amino acid sequences of rainbow trout (*Oncorhynchus mykiss*) lysozymes and their implications for the evolution of lysozyme and lactalbumin. *Journal of Molecular Evolution*, 32, 187-198.
https://doi.org/10.1007/BF02515392

De, P., Peng, Q., Traktuevc, D. O., Li, W., Yoder, M. C., March, K. L., Durdena, D. L., 2009. Expression of *RAC2* in endothelial cells is required for the postnatal neovascular response. *Experimental Cell Research*, 315, 248-263.
https://doi.org/10.1016/j.yexcr.2008.10.003

Dickinson, R. J., Keyse, S. M., 2006. Diverse physiological functions for dual-specificity MAP kinase phosphatases. *Journal of Cell Science*, 119, 4607-4615.
https://doi.org/10.1242/jcs.03266
Fernández-Trujillo, M. A., Porta, J., Manchado, M., Borrego, J. J., Alvarez, M. C., Béjar, J., 2008. c-Lysozyme from Senegalese sole (*Solea senegalensis*): cDNA cloning and expression pattern. *Fish & Shellfish Immunology*, 25, 697-700.
https://doi.org/10.1016/j.fsi.2008.08.004
Fortier, A. M., Riopel, K., Désaulniers. M., Cadrin, M., 2010. Novel insights into changes in biochemical properties of keratins 8 and 18 in griseofulvin-induced toxic liver injury. *Experimental & Molecular Pathology*, 89 (2), 117-125.
https://doi.org/10.1016/j.yexmp.2010.07.004
Hashimoto, K., Nakanishi, T., Kurosawa, Y., 1990. Isolation of carp genes encoding major histocompatibility complex antigens. *Proceedings of the National Academy of Sciences of the United States of America*, 87, 6863-6867.
https://doi.org/10.1016/10.1073/pnas.87.17.6863
Jiménezcantizano, R. M., Infante, C., Martinantonio, B., Ponce, M., Hachero, I., Navas, J. I., Manchado, M., 2008. Molecular characterization, phylogeny, and expression of c-type and g-type lysozymes in brill (*Scophthalmus rhombus*). *Fish & Shellfish Immunology*, 25, 57-65.
https://doi.org/10.1016/j.fsi.2007.12.009
Jung, S. J., Miyazaki, T., 2010. Herpesviral haematopoietic necrosis of goldfish, *Carassius auratus* (L.). *Journal of Fish Diseases*, 18, 211-220.
https://doi.org/10.1111/j.1365-2761.1995.tb00296.x
Kettle, A. J., Gedye, C. A., Winterbourn, C. C., 1993. Superoxide is an antagonist of antiinflammatory drugs that inhibit hypochlorous acid production by myeloperoxidase. *Biochemical Pharmacology*, 45, 2003-2010.
https://doi.org/10.1016/0006-2952(93)90010-T
Kong, S. Y., Jiang, Y. S., Wang, Q., Lu, J. F., Xu, D., Lu, L. Q., 2017. Detection methods of Cyprinid herpesvirus 2 infection in silver crucian carp (*Carassius auratus gibelio*) via a pORF72 monoclonal antibody. *Journal of Fish Diseases*, 40 (12), 1791-1798.
https://doi.org/10.1111/jfd.12648
Ku, N. O., Strnad, P., Zhong, B. H., Tao, G. Z., Omary, M. B., 2007. Keratins let liver live: Mutations predispose to liver disease and crosslinking generates Mallory-Denk bodies. *Hepatology*, 46 (5), 1639-1649.
https://doi.org/10.1002/hep.21976
Lau, D., Mollnau, H., Eiserich, J. P., Freeman, B. A., Daiber, A., Gehling, U. M., Brümmer, J., Rudolph, V., Münzel, T., Heitzer, T., 2005. Myeloperoxidase mediates neutrophil activation by association with CD11b/CD18 integrins. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 431-436.
https://doi.org/10.1073/pnas.0405193102
Lee, K. H., Lee, C. T., Kim, Y. W., Han, S. K., Shim, Y. S., Yoo, C. G., 2005. Preheating accelerates mitogen-activated protein (MAP) kinase inactivation post-heat shock via a heat shock protein 70-mediated increase in phosphorylated MAP kinase phosphatase-1. *Journal of Biological Chemistry*, 280, 13179-13186.
https://doi.org/10.1074/jbc.M410059200
Levraud, J. P., Boudinot, P., Colin, I., Benmansour, A., Peyrieras. N., Herbomel, P., Lutfalla, G., 2007. Identification of the zebrafish IFN receptor: implications for the origin of the vertebrate IFN system. *Journal of Immunology*, 178, 4385-4394.
https://doi.org/10.4049/jimmunol.178.7.4385
Lieschke, G. J., Oates, A. C., Crowhurst, M. O., Ward, A. C., Layton, J. E., 2001. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. *Blood*, 98, 3087-3096.
https://doi.org/10.1182/blood.V98.10.3087
Lim, M. B., Kuiper, J. W., Katchky, A., Goldberg, H., Glogauer, M., 2011. *Rac2* is required for the formation of neutrophil extracellular traps. *Journal of Leukocyte Biology*, 90 (4), 771-774.
https://doi.org/10.1189/jlb.1010549
Liu, Y. X., Wang, J., Guo, J., Wu, J., Lieberman, H. B., Yin, Y., 2008. *DUSP1* is controlled by p53 during the cellular response to oxidative stress. *Molecular Cancer Research*, 6 (4), 624-633.

https://doi.org/10.1158/1541-7786
Livak, K. J., Schmittgen, T. D., 2012. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25, 402-408.
https://doi.org/10.1006/meth.2001.1262
Luo, W., Zhang, J., Wen, J. F., Liu, H., Wang, W. M., Gao, Z. X., 2014. Molecular cloning and expression analysis of major histocompatibility complex class I, IIA and IIB genes of blunt snout bream (*Megalobrama amblycephala*). *Developmental & Comparative Immunology*, 42 (2), 169-173.
https://doi.org/10.1016/j.dci.2013.08.011
Majumdar, D., Tiernan, J. P., Lobo, A. J., Evans, C. A., Corfe, B. M., 2012. Keratins in colorectal epithelial function and disease. *International Journal of Experimental Pathology*, 93 (5), 305-318.
https://doi.org/10.1111/j.1365-2613.2012.00830.x
Minagawa, S., Hikima, J., Hirono, I., Aoki, T., Mori, H., 2001. Expression of Japanese flounder c-type lysozyme cDNA in insect cells. *Developmental & Comparative Immunology*, 25 (5-6), 439-445.
https://doi.org/10.1016/s0145-305x(01)00013-1
Pellinen, T., Arjonen, A., Vuoriluoto, K., Kallio, K., Fransen, J. A., Ivaska, J., 2006. Small GTPase Rab21 regulates cell adhesion and controls endosomal traffic of beta1-integrins. *Journal of Cell Biology*, 173 (5), 767-780.
https://doi.org/10.1083/jcb.200509019
Phung, T. T., Sugamata, R., Uno, K., Aratani, Y., Ozato, K., Kawachi, S., Thanh, N. L., Nakayama, T., Suzuki, K., 2012. Key role of regulated upon activation normal T-cell expressed and secreted, nonstructural protein1 and myeloperoxidase in cytokine storm induced by influenza virus PR-8 (A/H1N1) infection in A549 bronchial epithelial cells. *Microbiology & Immunology*, 55 (12), 874-884.
https://doi.org/10.1111/j.1348-0421.2011.00396.x
Pinto, R. D., Randelli, E., Buonocore, F., Pereira, P. J. B., Santos, N. M. S. D., 2013. Molecular cloning and characterization of sea bass (*Dicentrarchus labrax*, L.) MHC class I heavy chain and β2-microglobulin. *Developmental & Comparative Immunology*, 39 (3), 234-254.
https://doi.org/10.1016/j.dci.2012.10.002
Podok, P., Wang, H., Xu, L., Xu, D., Lu, L., 2014. Characterization of myeloid-specific peroxidase, *keratin 8*, and dual specificity *phosphatase 1* as innate immune genes involved in the resistance of crucian carp (*Carassius auratus gibelio*) to Cyprinid herpesvirus 2 infection. *Fish & Shellfish Immunology*, 41 (2), 531-540.
https://doi.org/10.1016/j.fsi.2014.10.001
Pradip, D., Peng. X., Durden, D. L., 2003. Rac2 specificity in macrophage integrin signaling: potential role for Syk kinase. *Journal of Biological Chemistry*, 278 (43), 41661-41669.
https://doi.org/10.1074/jbc.M306491200
Schutte, B., Henfling, M., Kölgen, W., Bouman, M., Meex, S., Leers, M. P., Nap, M., Björklund, V., Björklund, P., Björklund, B., 2004. Keratin 8/18 breakdown and reorganization during apoptosis. *Experimental Cell Research*, 297 (1), 11-26.
https://doi.org/10.1016/j.yexcr.2004.02.019
Sever, L., Vo, N. T., Lumsden, J., Bols, N. C., Dixon, B., 2014. Induction of rainbow trout MH class I and accessory proteins by viral haemorrhagic septicaemia virus. *Molecular Immunology*, 59 (2), 154-162.
https://doi.org/10.1016/j.molimm.2014.02.001
Shields, K. M., Panzhinskiy, E., Burns, N., Zawada, W. M., Das, M., 2011. Mitogen-activated protein kinase phosphatase-1 is a key regulator of hypoxia-induced vascular endothelial growth factor expression and vessel density in lung. *American Journal of Pathology*, 178, 98-109.
https://doi.org/10.1016/j.ajpath.2010.11.025
Simpson, J. C., Griffiths, G., Wesslingresnick, M., Fransen, J. A. M., Bennett, H., Jones, A. T., 2004. A role for the small GTPase Rab21 in the early endocytic pathway. *Journal of Cell Science*, 117 (Pt 26), 6297-6311.
https://doi.org/10.1242/jcs.01560
Strnad, P., Lienau, T. C., Tao, G. Z., Lazzeroni, L. C., Stickel, F., Schuppan, D., Omary, M. B., 2006. Keratin variants associate with progression of fibrosis during chronic hepatitis C infection. *Hepatology*, 43 (6), 1354–1363.
https://doi.org/10.1002/hep.21211

Wang, L., Zhou, Z., Guo, C., Rao, X., Xiao, J., Weng, S., Yin, Z., 2009. The alpha inhibitor of NF-kappaB (IkappaBalpha) from the mandarin fish binds with p65 NF-kappaB. *Fish & Shellfish Immunology*, 26 (3), 473-482.
https://doi.org/10.1016/j.fsi.2009.01.010
Wang, R., Feng, J., Li, C., Liu, S., Zhang, Y., Liu, Z., 2013. Four lysozymes (one c-type and three g-type) in catfish are drastically but differentially induced after bacterial infection. *Fish & Shellfish Immunology*, 35 (1), 136-145.
https://doi.org/10.1016/j.fsi.2013.04.014
Xi, Y., Zhang, Y., Shan, L., Liu, C., Zhe, J., Wang, Y., Ren, F., Chang, Z., 2012. *Rab21* attenuates EGF-mediated MAPK signaling through enhancing EGFR internalization and degradation. *Biochemical & Biophysical Research Communications*, 421 (4), 651-657.
https://doi.org/10.1016/j.bbrc.2012.04.049
Xia, S., Wang, H., Patorida, P., Xu, D., Jiang, Y., Lv, L, Motohiko, S., 2016. The expression analysis of immune genes and microscopic morphology of CCF cells in response to Cyprinid herpesvirus 2 infection. *Journal of Fisheries of China*, 40 (12), 1915-1922.
http://dx.doi.org/10.11964/jfc.20160110240
Xu, D., Xia, S., Podok, P., Xie, J., Lu, L., 2016. Characterization of *IκBa*, *Rab21* and *Rac2* as innate immune genes during infection with Aeromonas hydrophila and Cyprinid herpesvirus 2 in crucian carp (*Carassius auratus gibelio*). *Fish Pathology*, 51 (Special-issue), S7-S19.
https://doi.org/10.3147/jsfp.51.S79
Xu, L., Podok, P., Xie, J., Lu, L., 2014. Comparative analysis of differential gene expression in kidney tissues of moribund and surviving crucian carp (*Carassius auratus gibelio*) in response to cyprinid herpesvirus 2 infection. *Archives of Virology*, 159 (8), 1961-1974.
https://doi.org/10.1007/s00705-014-2011-9
Xu, T. J., Sun, Y. N., Cheng, Y. Z., Shi, G., Wang, R. X., 2011. Genomic sequences comparison and differential expression of miiuy croaker MHC class I gene, after infection by *Vibrio anguillarum*. *Developmental & Comparative Immunology*, 35 (4), 483-489.
https://doi.org/10.1016/j.dci.2010.12.003
Ye, X., Zhang, L., Tian, Y., Tan, A., Bai, J., Li, S., 2010. Identification and expression analysis of the g-type and c-type lysozymes in grass carp *Ctenopharyngodon idellus*. *Developmental & Comparative Immunology*, 34 (5), 501-509.
https://doi.org/10.1016/j.dci.2009.12.009