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## Functional characterisation of a Viperin knockout fish cell line

Lise Chaumont, Luc Jouneau, Mathilde Peruzzi, Pierre Boudinot, Bertrand Collet

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of top clonotype frequencies showed that pronephros and spleen B cells constitute distinct compartments with different IgH repertoires. Unexpectedly, after boost, the frequency of anti-VHSV clonotypes decreased both in pronephros and spleen, raising questions about B cell circulation. A better monitoring of B cell response kinetics in lymphoid tissues will be an essential step to understand B memory and plasma cell formation mechanisms in fish.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 104930**  
**CHARACTERIZATION OF COMPLEMENT FACTOR CD59 IN GOLDEN POMPANO (*TRACHINOTUS OVATUS*)**

Meiqin Mao, Zhenyu Lan, Yinhui Peng, Shaoyu Yang, Jiaxing He, Meng Le, Xing Lu, Jin Li, Peng Xu, Xinzhong Wu, Xiaohui Cai\*. *Guangxi Key Laboratory of Beibu Gulf Marine Biodiversity Conservation, Ocean College, Beibu Gulf University, Qinzhou, 535011, China*

\* Corresponding author

CD59, one of the essential inhibitors of the complement membrane attack complex (MAC), plays a crucial role in regulation of complement activation. In this study, the open CD59 cDNA (*ToCD59*) was successfully cloned and characterized from golden pompano (*Trachinotus ovatus*). The nucleotide sequence of *ToCD59* is 357 bp, encoding 118 amino acids, with a molecular weight of 13.09 kDa and an isoelectric point of 7.91. Prediction of protein domains showed that *ToCD59* contains a Lu domain, a C-terminal glycosylphosphatidylinositol (GPI) partial anchor and the three finger structures. Homology comparisons indicated that *ToCD59* share the highest sequence identity of 91.53% with *Paralichthys olivaceus* CD59, followed by 90.68% identity with *Miichthys miiuyi* CD59 and a relatively low identity of 89.83–88.14% with *Danio rerio*, *Epinephelus coioides*, *Cirrhinus mrigala*, *Ictalurus punctatus* and *Takifugurubripes* CD59. The result of phylogenetic tree analysis showed that *ToCD59* was clustered together with the teleosts CD59, and separating from mammalian CD59. The subcellular localization of *ToCD59* revealed that it is mainly distributed in the membrane and cytoplasm, somewhat aggregated around the nucleus, and very little inside the nucleus in A549 cells. The tissue distribution of *ToCD59* in healthy golden pompano showed that *ToCD59* is ubiquitously expressed in all analyzed tissues, with the highest expression level in brain and followed by liver. After infection with *Streptococcus agalactis* and *Vibrio alginolyticus*, the expression level of *ToCD59* was significantly up-regulated in the head, kidney, liver, gill, brain but down-regulated in the spleen. This may be related to the fact that the CD59 protein acts as an inhibitor to prevent normal cell damage when the complement system activates cytolysis. Taken together, the results indicated that *ToCD59* may be involved in immune regulation of the golden pompano against bacteria, which lays a foundation for further study on the complement regulation mechanism in teleost fish.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 104931**  
**EVOLUTIONARY CONSERVED MECHANISMS REGULATING STRESS-INDUCED NEUTROPHIL REDISTRIBUTION IN FISH.**

Katarzyna Klak<sup>1,2</sup>, Magdalena Maciuszek<sup>1</sup>, Mikolaj Mazur<sup>1,2</sup>, Magdalena Marcinkowska<sup>1,2</sup>, Lukasz Pijanowski<sup>1</sup>, Joanna Homa<sup>1</sup>, B.M. Lidy Verburg-van Kemenade<sup>3</sup>, Krzysztof Rakus<sup>1</sup>, Magdalena Chadzinska<sup>1,\*</sup>. <sup>1</sup>Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, PL30-387, Krakow, Poland; <sup>2</sup>Doctoral School of Exact and Natural Sciences, Jagiellonian University, Łojasiewicza 11, PL30-348, Krakow, Poland; <sup>3</sup>Cell Biology and Immunology Group, Department of Animal Sciences, Wageningen University, P.O. Box 338, 6700 AH, Wageningen, The Netherlands

\* Corresponding author

Stress may pose a serious challenge to the immune homeostasis. Stress however also may prepare the immune system for challenges such as wounding or infection, which are likely to happen during a fight or flight

response. In common carp (*Cyprinus carpio*) we studied the involvement of CXC receptors, stress hormone receptors and microbiota in stress-induced neutrophil redistribution. It was determined by an *in vivo* study with selective CXCR inhibitors and GR/MR, ADR and MC2R antagonists during restraint stress. The stress induced neutrophil redistribution from hematopoietic organs into the blood circulation. This increase was cortisol-induced and GR-dependent and was decreased in animals with antibiotic-induced depletion of gut microbiota. Moreover, stress upregulated the expression of genes encoding CXCL12 and CXCL8 chemokines, their receptors, and GCSFR and MMP9 while blocking of the CXCR1, 2 and 4 receptors with selective inhibitors decreased the stress-induced neutrophil redistribution and affected the expression of genes encoding CXC chemokines and CXCRs as well as GCSFR and MMP9. Altogether, our data demonstrate that acute stress leads to mobilization of the immune system, characterized by neutrophilia. CXC chemokines, and CXC receptors are involved in this stress-induced redistribution of PMNs from the hematopoietic tissue into the peripheral blood. This phenomenon is directly regulated by interactions between cortisol and the GR/MR as well as microbiota.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 104932**  
**FUNCTIONAL CHARACTERISATION OF A VIPERIN KNOCKOUT FISH CELL LINE**

L. Chaumont\*, L. Jouneau, M. Peruzzi, P. Boudinot, B. Collet. *University of Paris-Saclay, INRAE, UVSQ, VIM, 78350, Jouy-en-Josas, France*

\* Corresponding author

The antiviral response is mediated through the expression of a wide range of interferon-stimulated genes (ISGs). The radical S-adenosyl-methionine domain-containing protein 2 (Rsd2), also known as Viperin, is a well-known ISG-encoded protein with a potent antiviral activity across species and cell types. In mammals, Viperin exerts its function through different pathways: (1) it catalyses the conversion of cytidine triphosphate (CTP) to its analogue ddhCTP, which inhibits the replication of RNA viruses; (2) Viperin also interacts with host proteins involved in innate immune signalling and in metabolic pathways exploited by viruses during their life cycle (e.g. cholesterol biosynthesis). In order to understand the role of fish Viperin in the antiviral response, we developed a fathead minnow (*Pimephales promelas*) clonal cell line in which the *viperin* gene has been knocked out by CRISPR/Cas9 genome-editing. The knockout cell line was validated by gDNA sequencing and by western blot upon stimulation with poly(I:C). In order to achieve a global overview of the transcriptional response between the knockout and the wildtype cell lines, we performed a comparative RNA-seq analysis of the whole transcriptome of the two cell lines with or without a 24h-long stimulation with recombinant fathead minnow type I interferon. Our results show that core ISGs are not differentially modulated in the knockout cells compared to their parental counterparts, suggesting that Viperin does not exert a positive feedback on the interferon response in these cells. However, functional enrichment analysis indicates that Viperin might be involved in the regulation of actin skeleton and cholesterol metabolism.

**DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY 148 (2023) 104922 104933**  
**INTERFERON SIGNALING PATHWAY MODULATED BY TEMPERATURE IN GROUPER IMMUNE SYSTEM**

Tzong-Yueh Chen\*, Zhi Yan Chong, Yuan-Ning Wang, Hong Ye Ong, Ting-Yu Wang. *Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan; University Center for Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan; Translational Center for Marine Biotechnology, National Cheng Kung University, Tainan, Taiwan; Agriculture Biotechnology Research Center, National Cheng Kung University, Tainan, Taiwan*

\* Corresponding author

The objective of this study was to investigate the impact of temperature on the signaling pathway of interferons (IFNs) and the immune system