Keywords: Inhibitory factors; NF-kB; NKIRAS; Rainbow Trout; Furuncolosis

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O-102.

An improved genome assembly for *Larimichthys crocea* reveals hepcidin gene expansion with diversified regulation and function

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Abstract
Larimichthys crocea (large yellow croaker) is a type of perciform fish well known for its peculiar physiological properties and economic value. Here, we constructed an improved version of the L. crocea genome assembly, which contained 26,100 protein-coding genes. Twenty-four pseudo-chromosomes of L. crocea were also reconstructed, comprising 90% of the genome assembly. This improved assembly revealed several expansions in gene families associated with olfactory detection, detoxification, and innate immunity. Specifically, six hepcidin genes (LcHamps) were identified in L. crocea, possibly resulting from lineage-specific gene duplication. All LcHamps possessed similar genomic structures and functional domains, but varied substantially with respect to expression pattern, transcriptional regulation, and biological function. LcHamp1 was associated specifically with iron metabolism, while LcHamp2s were functionally diverse, involving in antibacterial activity, antiviral activity, and regulation of intracellular iron metabolism. This functional diversity among gene copies may have allowed L. crocea to adapt to diverse environmental conditions.
Keywords: *Larimichthys crocea*, Genome, Hepcidin

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O-103.

Role of sea lice secretome in host-parasite interaction: Immune modulation of SHK-1 cells exposed to *Caligus rogercresseyi* secretome

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Abstract

Caligus rogercresseyi is an ectoparasite that feeds on mucus, blood, and skin of its host. For a successful infestation, sea lice secrete proteins that allow avoiding host response. Among them, it has been described that trypsin and chymotrypsin have strong proteolytic activity in the peritrophic matrix of the intestinal parasite. In relation to the copepod *Lepeophtheirus salmonis*, it has been suggested that one of the strategies to successfully parasitize its host is given by the secretion of molecules such as proteases, prostaglandin synthetase E2 (PGE2) and cathepsin, causing immunodepression in fish. Moreover, from transcriptomic studies of *C. rogercresseyi* developmental stage has been identified secretome-related proteins as cathepsin, trypsin, and serpin highly regulated during the infective stage, copepodid. The aim of this study was to evaluate the effects of *C. rogercresseyi* secretome over salmon immune and stress response by an in vitro approach using SHK1 cell line. Proteins identification and characterization were performed using the transcriptome database of *C. rogercresseyi*. Characterized sequences were cloned into an expression vector, pET30a and expressed in *Escherichia coli* system. Recombinant proteins were purified by His-tag affinity chromatography. SHK1 cell line was stimulated with 25 ng/mL, 50 ng/mL and 100 ng/mL of recombinant proteins for 24 hours. After cells stimulation, cells were collected for RNA extraction for immune-related genes expression analysis by RT-qPCR. A total of two isoforms of cathepsin, serpin, and trypsin were characterized. With a molecular weight of 36.4, 36.3, 43.5, 49, 26.4 and 27 kDa, respectively. After 24 h of stimulation cell damage was observed in all groups exposed to secretome proteins. Furthermore, differences in immune-related genes expression levels were observed among cells exposed to secretome proteins and control group. This study provides novel information associated with host-parasite interactions associated with *C. rogercresseyi* secretome effects on salmon.
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Keywords: Interaction parasite-host, *Caligus rogercresseyi*; secretome, SHK1 cell line, RT-qPCR

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O-104.

TLR-mediated type-I interferon production and the regulatory mechanisms in carp thrombocytes

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Abstract
In early stage of viral infection, type-I interferons (IFNs) are produced by signaling from innate immune receptors such as Toll-like receptors (TLRs), which recognize virus-specific molecular patterns including nucleic acids. The type-I IFN transcriptions induced by TLRs are regulated via nuclear factor κB (NF-κB) complex and interferon regulatory factors (IRFs), but these details are still unclear in fish. In the present study, we show that thrombocytes in common carp (*Cyprinus carpio*) have a potent ability to produce large amount of IFNs in response to TLR signaling. Magnetic-sorted HB-8 mAb+ carp thrombocytes and negatively sorted other peripheral blood leukocytes (PBLs) were incubated with resiquimod (also called R848, a potent agonist of TLR7/8), followed by qPCR analysis. The expression levels of the common carp type-I IFNs (ccIFN1 and ccIFN2) in thrombocytes were considerably higher compared with that of in other PBLs. Whereas the ccIFN1 expression was relatively lower than the ccIFN2, the R848 stimulant highly upregulated the ccIFN1 expression than ccIFN2. Although typical inflammatory cytokines including interleukin-6 were also upregulated in thrombocytes, the expression levels were still lower than those in other PBLs. These results indicate that activation of carp thrombocytes by R848 inclines immune system toward antiviral response, rather than inflammation. Expression levels of IRF3 and IRF7 were also upregulated by R848, implying that the IFN transcriptions were activated by these IRFs. The expression of the IFNs and inflammatory cytokines were decreased by several NF-κB signaling inhibitors such as BAY11-7082 or phenethyl caffeate, however, sensitivities to each inhibitor were different between the IFNs and other cytokines. In the presence of those inhibitors, the ccIFN2 expression was correlated with the level of IRF3. In contrast, ccIFN1 expressions seem to be linked to IRF7, suggesting that these two IRFs regulates different IFN genes separately. Our finding suggests that fish thrombocytes are important components for antiviral immunity and can

be a new target for the strategy of disease control and vaccine development.

Keywords: Thrombocytes, Interferon, TLRs, innate immunity, interferon regulatory factor

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O-105.

Activation of DExD/H-box RNA helicases during infection of zebrafish and common carp with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV)

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Abstract
The innate immune system detects viral infection predominantly by sensing viral nucleic acids in infected cells. The main sensors of viral RNA in cytoplasm are members of RIG-I-like receptors (RLR) such as RIG-I, MDA5 and LGP2. Recently, several non-RLR DExD/H-box RNA helicases have been also shown to play important role in sensing of viral nucleic acids in the cytoplasm and to activate downstream signaling pathways leading to type I interferons (IFN) production and anti-viral response in mammals. However, the mechanisms of action of these RNA helicases are still not fully understood, and their role in the anti-viral immune response in fish has not been studied. In the present work we aimed to study, for the first time in fish, the anti-viral role of DExD/H-box RNA helicases: DDX1, DDX3, DHX9, DDX21 and DHX36 during viral infection of two cyprinid fish: zebrafish (*Danio rerio*) and common carp (*Cyprinus carpio*). We studied expression of DExD/H-box RNA helicases, type I IFNs and antiviral proteins in zebrafish during infection with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV) both *in vitro* (ZF4 cell line) and *in vivo*. Moreover, expression of studied genes was analyzed in common carp during *in vivo* infection with SVCV. *In vitro* studies of both viral models demonstrated a significant up-regulation of the expression of IFN type I genes in ZF4 cell line. However, SVCV did not induce changes in the gene expression of DExD/H-box RNA helicases, up-regulation of the expression of *ddx3*, *dhx9* and *ddx21* was observed in ZF4 cells upon CSV infection. *In vivo* SVCV infection of zebrafish induced a significant up-regulation of *ddx1* and *dhx36* expression while CSV infection induced a significant up-regulation of *ddx1* and *dhx9* expression. In both infection models an up-regulation of the expression of IFN type I genes and interferon stimulated genes (ISG) *mxa* and *vig-1* was observed. In common carp SVCV infection resulted in up-regulation of the expression of *ddx1*, *dhx9* and *ddx21*, IFN type I and *vig-1*. In both zebrafish and common carp, the up-regulation of the gene expression of DExD/H-box RNA helicases correlated with the increase of the viral load and in most of the cases preceded up-regulation of the IFN type I genes expression. In conclusions our data suggest that non-RLR DExD/H-box RNA helicases might be involved in fish in sensing of viral infection and induction of anti-viaral immune response.
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Keywords: DExD/H-box RNA helicases, interferons, *vig-1*, SVCV, CSV

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O-106.

The fish coagulation system could help to prevent infection by the ciliate parasite *Philasterides dicentrarchi*

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Abstract
In addition to its role in hemostasis, the coagulation system is involved in defence against pathogens in invertebrates and vertebrates. In mammals, the coagulation system has been shown to participate in entrapping pathogens and activating the early immune response. Although many studies have described different components of the fish coagulation system, there is a lack of information about the importance of the system in host defence against pathogens. In the present study, we showed that injecting turbot (*Scophthalamus maximus*) with the pathogenic ciliate *Philasterides dicentrarchi* generates the formation of macroscopic intraperitoneal clots in the fish. The clots contained abundant, immobilized ciliates, many of which were lysed. We observed that the plasma clots immobilize and kill the ciliates in vitro. However, fish plasma treated with a tetrapeptide known to inhibit fibrinogen/thrombin clotting in mammals killed *P. dicentrarchi* slightly faster than the untreated plasma, although the overall mortality rate was similar. We also found that kaolin, a particulate activator of the intrinsic pathway in mammals, accelerates plasma clotting in turbot. PMA-stimulated neutrophils, living ciliates and several ciliate components (such as cilia, proteases and DNA) also displayed procoagulant activity in vitro. In addition to generating clots in the peritoneal cavity, i.p. injection of ciliates generated massive migration of neutrophils to the peritoneal cavity, with the formation of large cell aggregates and of numerous fibrin-like fibres in the peritoneal exudate, many of which were associated with peritoneal leukocytes and ciliates. Expression of the CD18/CD11b gene, an integrin associated with cell adhesion and the induction of fibrin formation, was upregulated in the peritoneal leukocytes. In conclusion, the results of the present study suggest that the fish coagulation system plays an important role in immobilizing *P. dicentrarchi* during early moments of infection and appears to be an important component of the protection against this pathogen in fish.
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Keywords: Coagulation system; Complement; Fish; Neutrophils; *Philasterides dicentrarchi*; Plasma

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O-107.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is lethal to *Flavobacterium psychrophilum* through membrane permeabilization and by priming immune function in rainbow trout macrophages

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