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O-029.

Macrophage polarization in fish transcriptional profiles and metabolic changes

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Abstract
Macrophages of higher vertebrates can display a range of functional phenotypes, while the chief M1 and M2 activation states appear to operate under the guidance of primordially-conserved principles. We have been studying the evolutionary conservation of these M1 and M2 macrophage activation states in teleost carp, mainly by measuring functional responses such as nitric oxide production (M1) and arginase activity (M2). However, the picture of M1 and M2 activation states in teleosts is still far from complete. To complement our understanding of teleost macrophage polarization we first studied activation-state specific gene expression profiles through an unbiased whole transcriptome approach in addition to functional assays. Secondly, we studied the conservation of bioenergetic and metabolic pathways paramount to activation-state specific functions.
Here we report differential transcriptional profiles for M1 (LPS stimulated) and M2 (exogenous cAMP stimulated) carp macrophages and discuss the conservation of these profiles, which include multiple conserved markers. In addition, we show an enhanced M1 profile when IFN-γ is combined with LPS.
Although essential to direct and support macrophage activation-state specific functions, conservation of bioenergetic and metabolic pathways have not been studied in detail in polarized carp macrophages. Generally, mammalian M1 macrophages show relatively high glycolysis rates while M2 macrophages are geared towards oxidative phosphorylation to generate energy. We studied whether the enhancement of these specific energy metabolism pathways is conserved. We optimized for carp macrophages the determination of cellular oxygen consumption rate (OCR) as a measure for oxidative phosphorylation, and the determination of extracellular acidification rate (ECAR) as a measure for glycolysis, using the Seahorse real-time Mito Stress Test. We have gained insight in the energy metabolism pathways utilized by carp macrophages driven to M1 or M2 activation states by using specific parameters measured with this test. Both our whole transcriptome approach and assays to measure bioenergetic and metabolic pathways provide valuable additions to studies addressing the evolutionary conservation of M1 and M2 macrophage activation states.
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Keywords: Macrophage, polarization, transcriptome, metabolism, immunometabolism

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O-030.

Advantages and disadvantages of zebrafish as a model of inflammation

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Abstract
Zebrafish (*Danio rerio*), largely used as a model for studying developmental processes, has also emerged as a valuable system for modelling human disease. Zebrafish possesses a complex immune system comparable to those of mammalian models. Nevertheless, whole-genome duplication event and subfunction specialization of gene duplicates results in a more intricate relationship among the components implicated in the immune response and as a consequence in the inflammatory response. This is the case of the of the complement component *c3* with 8 different genes in the zebrafish genome and different functions. This aspect, that could be considered an inconvenient, contrasts with the clear advantages that this model offers. We show how the real-time imaging and the use of the whole animal are excellent tools to visualize the *in vivo* interaction of a pathogen with the immune system. Also, we demonstrate how the genomic responses of adult zebrafish tissues can effectively reproduce the mammalian inflammatory process induced by acute endotoxin stress. Immune signalling has been well conserved throughout evolution and zebrafish and mammal genomic responses after lipopolysaccharide stimulation are highly correlated. Therefore, we confirm that zebrafish is an ideal model to study the basic mechanisms of inflammation and to model human inflammatory diseases.
Keywords: Zebrafish, inflammation, imaging, complement, transcriptome

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O-031.

Viral resistance and interferon signalling in signal transducer and activator of transcription (STAT)-1 and STAT2 knockout salmonids cells

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Abstract
Interferons (IFNs) belong to a group of cytokines specialised in the immunity to viruses. Upon viral infection, type I IFN is produced and alters the transcriptome of responding cells through induction of a set of Interferon Stimulated Genes (ISGs) with regulatory or antiviral function, resulting in a cellular antiviral state. Fish genomes have both type I IFNs and type II IFN (IFNγ), but type III (λ) IFN has not been identified in fish. The mechanisms of the downstream signalling remain partly undefined. In mammals, members of the Signal Transducer and Activator of family of transcription (STAT) factors are responsible for the transmission of the signal from cytokine receptors, and STAT2 is required for type I but not type II IFN signalling. In fish, the role of STAT2 in IFN signalling remains unclear. Using CRISPR/Cas9 genome editing, we generated two Chinook salmon (*Oncorhynchus tshawytscha*) cell lines with transcription factors STAT1 and 2 knocked out. GS2 and GS1A are stat2-/- and stat1a1-/- stat1a2-/- stat1b1+/+ stat1b2+/+, respectively. In these cell lines, the induction of ISGs by stimulation with a recombinant type I IFN is completely obliterated as evidenced by RNA-seq analysis of the transcriptome and/or qPCR gene expression profiling in comparison with the wild type parental cell line. In contrast, the type II IFN signalling pathway is obliterated only in GS1A but not in GS2. Despite a complete absence of ISGs

induction, the GS2 and GS1A cell lines still have a remarkable ability to inhibit viral replication. Therefore, other STAT1/2-independent pathways may be induced by the viral infection, potentially illustrating the robustness and redundancy of the innate antiviral defences in fish.
Keywords: Chinook salmon, CRISPR/Cas9, STAT2, STAT1, interferon signalling

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O-032.

Phylogeny and expression of the tetraspanin CD9 in salmonid cell lines in response to interferon stimulation

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Abstract
CD9 is a member of the cell membrane associated tetraspanin family and has been shown to have a wide array of functions, including promotion of MHC clustering, antigen presentation, T cell activation, cell adhesion, motility, growth and differentiation, signal transduction, tumor formation and egg/sperm fusion. CD9 is ubiquitously expressed in mammalian tissues and its roles are cell type dependent. CD9 is a typical interferon stimulated gene and further associated with MHC II and the immune system and inflammation in general, as has been shown in mammals and to a lesser extent in fish. In mammals, some viruses, such as influenza, coronavirus and hepatitis C, exploit CD9 for exit of new virus particles from host cells. In contrast, increased expression of CD9 can limit HIV-I virus budding.
Due the limited knowledge of the involvement of CD9 in immune system responses in fish, we explored the phylogeny and expression of this gene in salmonids. We found 6 paralogues, which can be further organized into three distinct clades. We termed these clades CD9a, CD9b and CD9c, each of which include two paralogues reflecting the salmonid specific whole genome duplication. CD9a and CD9b are closely related and have the greatest sequence homology with the mammalian single copy gene of CD9, indicative of the teleost specific whole genome duplication. The CD9c clade is very distinct to CD9a and CD9b in sequence identity and further shows little sequence homology with the mammalian CD9, therefore could be an ancestral form of CD9 that was subsequently lost in all other vertebrate classes.
We investigated the expression of the different paralogues in embryonic chinook salmon cells (CHSE) stimulated with interferon type I, an inducer of the antiviral pathways in fish.
The paralogues of clade CD9c were highly inducible by interferon stimulation, whilst CD9a and CD9b appeared to be non-responsive. The specific inducibility of the ancestral CD9c clade to interferon type I highlights the unique immune responses in teleost. The presence of 6 paralogues organized in three clades may also reflect the diversity of roles this gene has been implicated in. In future, we aim to explore the expression of CD9, especially the putatively immune system relevant clade CD9c, in different cell types at baseline and in response to virus stimulations.
This study contributes to a better understanding of CD9 involvement in immune system responses and how the gene is related to the antiviral interferon type I response. As CD9 has been shown to be important for the replication of certain viruses in mammals, this could be explored for fish viruses and potentially used as an anti-viral target.
Keywords: Tetraspanins, Salmonid, Interferon signaling, Antiviral immune response, Phylogeny

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O-033.

Genomics for the understanding of the host-pathogen interaction: the case of the Atlantic salmon and *Piscirickettsia salmonis*

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Abstract
During an infection both host and pathogen undergo a deep transcriptomic remodeling that will orchestrate either the pathogen clearance or host infection. These changes involve both the regulation of protein coding genes (mRNA) and non-coding RNAs (ncRNAs) elements, such as lncRNAs and miRNAs. Thus, knowing how these elements are modulated can reveal key aspects about hostpathogen interaction. Through RNA-seq, miRNA-seq and dual RNA-seq, we explored the coding and non-coding transcriptional response in Atlantic salmon infected with the intracellular bacterium Piscirickettsia salmonis. Differential expression analysis revealed that fish respond to P. *Salmonis* infection through modulation of different coding genes associated with immunity, clathrin mediated endocytosis and iron metabolism responses. In addition, a strong response associated with ncRNAs was also evidenced. Our results suggested that these ncRNAs might fulfilling key regulatory roles in the response of the Atlantic salmon to *P. salmonis* infection. On the other hand, bacteria transcriptomic response was associated with a large number of genes involved in amino acid metabolism. Genome wide comparison and in vitro studies evidenced a metabolic dependency of *P. salmonis* on salmon amino acids. Based in our results, we propose that amino acids might be an important component of the nutritional immunity triggered by the Atlantic salmon to cope with *P. salmonis* infection. Overall, our results evidence how genomics can lead us to the understanding of novel means of interaction between host and pathogens in marine models.
Keywords: Dual RNA-Seq, Piscirickettsia salmonis, Atlantic salmon, Nutritional immunity, metabolic dependency, amino acids

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O-034.

***In vitro* rainbow trout transcriptome reveals immune evasion associated with higher virulence of viral haemorrhagic septicaemia virus**

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Abstract
Rainbow trout pathogenic freshwater viral haemorrhagic septicaemia virus (VHSV) emerged from an ancestral marine virus, however the pathogenic mechanism of the virulent freshwater VHSV remains unknown. In the present work, the transcriptome of RTG-2 cells inoculated with two pathogenic (J167 and DK-5131) and two non-pathogenic (96-43/8 and 1p49) isolates were analyzed at 3, 6, and 12 hours and compared to control samples using RNA-seq. Although VSHV isolates showed the same pattern of viral replication, the transcriptomic profiles in RTG-2 cells were dramatically different between pathogenic and non-pathogenic isolates, revealing a lack of sensing of the viral replication in cells inoculated with both pathogenic VHSVs at early stages of infection. Functional annotation analysis of differentially-expressed genes between non-pathogenic VHSV and controls revealed an enrichment of pathways involved in the defense to biotic stimulus and metabolic processes (strong up-regulation of genes), and lipid metabolism and cell cycle (down-regulation of genes) In contrast, cholesterol and cytoskeleton mobility pathways were enriched (up-regulation of genes) by both pathogenic VHSV. Furthermore, an increasingly