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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