EPIGENETIC STUDY OF FISH ERYTHROCYTES FOR THE SEARCH OF INNOVATIVE ANTIVIRAL SOLUTIONS IN AQUACULTURE

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Summary

Nucleated erythrocytes (RBCs), characteristic of fish, amphibians, reptiles and birds, are multifunctional cells, as in addition to participating in gas exchange and transport, they have also been described to respond to various pathogens, including viral infections[1,2].

On the other hand, recent studies in immunology have pointed to a process called trained immunity[3] which is defined as the long-term functional reprogramming of cells of the innate immune system as well as other blood cells such as RBCs, induced by exogenous or endogenous challenges, leading to an enhanced response to a second challenge after a return to a non-activated state. Trained immunity is mediated by epigenetic reprogramming[4].

In the present work, rainbow trout, an important species in salmonid aquaculture, will be used as a model species that will be subjected to various stimuli such as: the viral hemorrhagic septicemia virus (VHSV)[5], a DNA vaccine (pGVHSV)[6], and an antiviral treatment based on green tea (*Camellia sinensis*)[7]. With this working model exposed to these stimuli, we will evaluate the role of epigenetic reprogramming of RBCs.

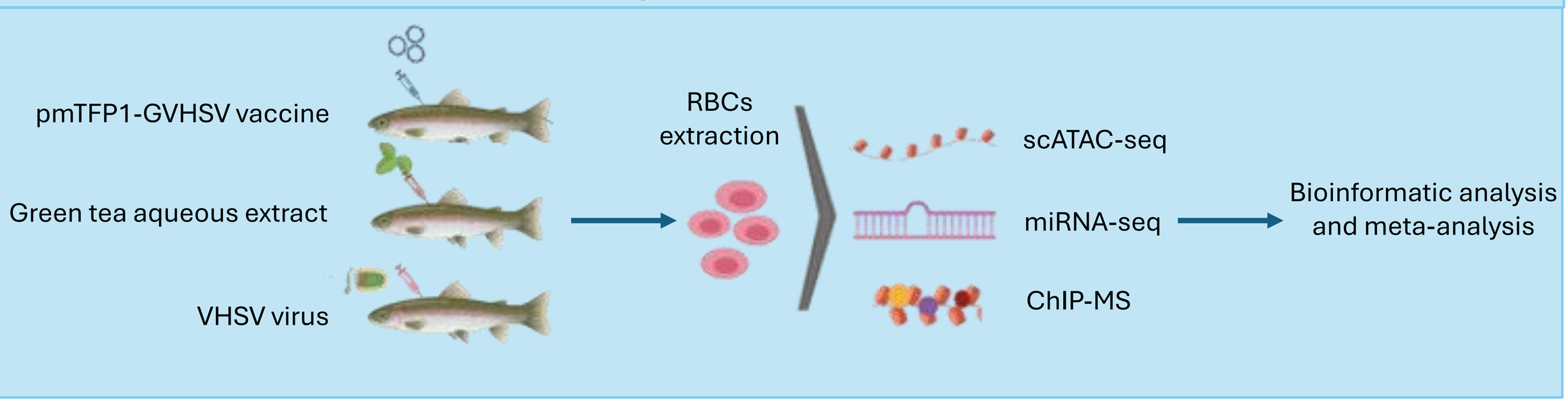
Our aim is to evaluate the epigenetic reprogramming of RBCs from fish exposed to different prophylactics and therapeutics by means of different epigenetic techniques: (i) study of epigenetic modifications by scATAC-seq; (ii) evaluation of the miRNA-ome; and (iii) analysis of the DNA-bound proteome by chromatin immunoprecipitation (ChIP) with mass spectrometry (MS).

The expected results will determine the differential gene expression of genes involved in the immune response in those RBCs exposed to the aforementioned stimuli. These results could lead to the development of new therapeutics and prophylactics in salmonid aquaculture.

Objectives

- ✓ Evaluating the epigenetic reprogramming of Red Blood Cells (RBCs) of rainbow trout exposed to different treatments.
- ✓ Identifying new pharmacological targets or biomarkers related to infectious processes in aquaculture.

Working model and workflow



scATAC-seq

Chromatin accesibility will be evaluated in rainbow trout RBCs using BD Rhapsody technology. Then, libraries will be sequenced.

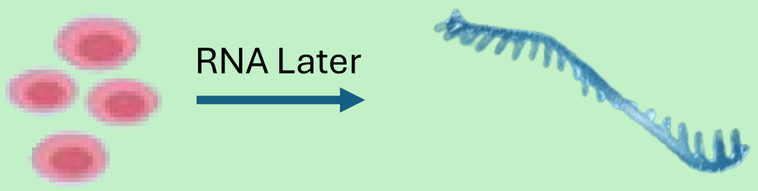
BD Rhapsody cartridge

BD Rhapsody HT Xpress

<u>Objective</u>: Identifying, at single cell level, open chromatin regions in rainbow trout RBCs exposed to the different treatments.

miRNA-seq

RBCs samples will be preserved in RNA Later and sequenced.



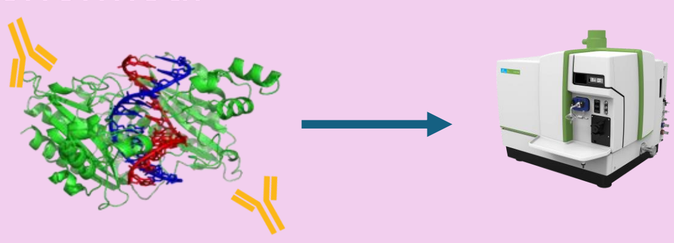
RBCs

miRNA sequencing

<u>Objective</u>:Comparing miRNAome expression patterns in rainbow trout RBCs exposed to the different treatments.

ChIP-MS

DNA-bound proteome will be crosslinked and ChIP assay and proteomic analysis will be performed.



Chromatin Inmunoprecipitation

Proteomic analysis

<u>Objective</u>: Identification of methylated and acetylated histone-associated proteins, such as transcription factor in rainbow trout RBCs exposed to the different treatments.

Expected results

- Optimizing new avenous of research in terms of epigenetic reprogramming in aquaculture in order to fight infectious processes.
- The development of new profilactic/therapeutic or biomarkers tools as well as cheaper administration router that avoid economic lost in aquaculture.

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Acknowledgements

This study is supported by MCIN-AEI CNS2022-135920 project.