Universidad de Navarra
Facultad de Ciencias

MASTER'S DEGREE IN BIOMEDICAL RESEARCH
Research Project Proposal
Academic year 2023-2024

Project Nº 45

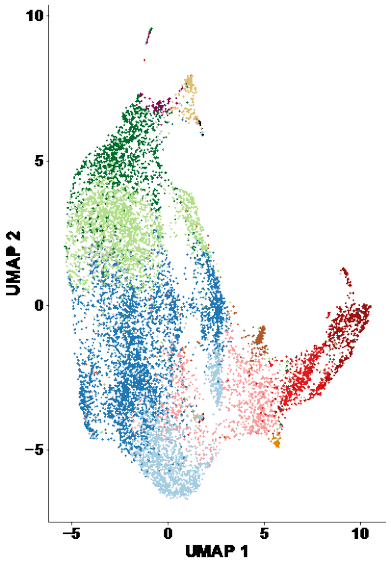
Title: Identification of gene regulatory networks guiding aberrant hematopoiesis in myeloid malignancies using single-cell RNA sequencing

Department/ Laboratory: *Myeloid Malignancies, Laboratory 1.04, Hematology-Oncology Program, CIMA Universidad de Navarra*

Director 1 *Teresa Ezponda Itoiz*
Contact: *tezponda@unav.es*

Summary:

Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) represent hematological malignancies characterized by alterations in the differentiation and proliferation of hematopoietic progenitor cells. Genetic alterations do not fully explain the molecular pathogenesis of these diseases, indicating that other types of lesions, such as transcriptional alterations, may play a role in its development. In order to fully characterize the transcriptional lesions guiding aberrant hematopoiesis in these patients, our group has started with the analysis of early hematopoietic progenitors of MDS and AML patients and age-matched healthy donors. To do so, we use the state-of-the-art technology single-cell RNA sequencing (scRNAseq), which allows for the characterization of the transcriptome of single cells, allowing for the detection of all clusters of cells representing undifferentiated hematopoitic stem cells (HSCs) and progenitor cells of different cell lineages. These analyses allow us to identify gene regulatory networks (GRNs), which represent transcription factors and associated target genes, with altered activity in the patients. Such GRNs may be of great relevance, as they can act as master regulators of the transcriptome, and thus the phenotype of a cell.



U-AMP showing the main clusters observed for single-cell RNA-seq data performed in hematopoietic cells of an MDS patient. Each dot corresponds to a cell and each color to a group of cells with similar expression profiles, representing different subpopulations (HSCs, erythroid progenitors, neutrophil progenitors…etc)

The aim of the present project is to determine the role of altered GRNs in the phenotype of MDS and AML. Firstly, the candidate will learn how to perform scRNAseq from bone marrow primary samples. Secondly, the student will interrogate the potential functional involvement of candidate GRNs in the promotion of an MDS/AML phenotype. To do so, the student will use an ex-vivo myeloid differentiation system starting from primary cells (HSCs from healthy donors). This system allows us to model early stages of hematopoietic differentiation in vitro, where the expression of specific genes can be manipulated. The candidate will

generate lentiviruses to overexpress the factor of interest and use the differentiation system and flow cytometry analyses to evaluate the effect of the altered GRN on normal hematopoietic differentiation.

All in all, the candidate will acquire the following expertise:

- State of the art transcriptome profiling techniques: including single cell transcriptome profiling by scRNA-seq analyses and bulk transcriptome profiling of small cell populations by low input RNA-seq.
- Human hematopoiesis cell biology.
- HSCs isolation, differentiation and culture.
- Ex-vivo myeloid differentiation system.
- Cloning and production of lentiviruses.
- Flow cytometry analyses of hematopoietic differentiation

yes	
no	x

Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?