Research Project Proposal
Academic year 2021-2022
Máster en Investigación Biomédica

Project Nº 50

Title: Identification of transcriptional lesions guiding aberrant hematopoiesis in myelodysplastic syndromes using single-cell RNA sequencing

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

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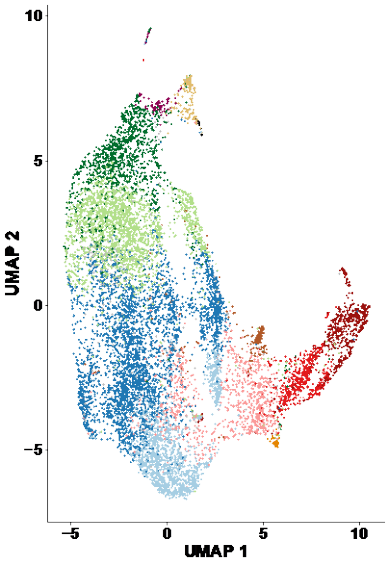
Summary:

Myelodysplastic syndromes (MDS) represent age-related hematological malignancies characterized by alterations in the differentiation and proliferation of hematopoietic progenitor cells. Genetic alterations do not fully explain the molecular pathogenesis of the disease, 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 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. Analysis of differentiation trajectories (i.e: from HSC to erythroid cells) in these data allow to determine genes with aberrant patterns of expression across the differentiation and commitment to specific hematopoietic cell lineages. Such genes, could have a direct role in promoting aberrant differentiation phenotypes.



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 one of these candidate genes in the phenotype of MDS. 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 genes in the promotion of an MDS 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 upregulation of the factor on normal hematopoietic differentiation. Furthermore, to determine the transcriptional lesions promoted by the upregulation of the factor, the candidate will perform RNA-seq analyses upon its exogenous overexpression in primary cells.

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?