ID No.	K2010
Project Title	Molecular signature of hematopoietic aging
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Report

Scientific Context and Principal Objectives

The aging of the hematopoietic system is characterized by immunosenescence and an increase in disease related to myeloid cells. These age-related alterations of the hematopoietic compartment are thought to come from the pool of old hematopoietic stem cells (HSCs) that alter and drift upon the environmental cues they perceive overtime. By comparing, at the single cell level, the molecular (transcriptional and epigenomic) and cellular changes of HSCs in several mouse models of aging, **we aim to reveal the elements that define the heterogeneity of the HSC pool, the clonal selection of aging and its link with inflammation and leukemia onset.** The integration and modelling of these elements will identify potential intervention points that will be validated in a functional way. Our study will lead to molecular characterization of HSC aging processes, a better understanding of their effect on HSC deterioration and clonal drift, and the identification of key points to address the deleterious effects of aging.

Data collected within the first year of the IMSUT project

To detect early events in the HSC differentiation process, we have generated and analysed 15000 RNAseq from either young (2 months) or old (18 months) wild type HSCs, using droplet single-cell RNA-Seq (10x Genomics Chromium). **We revealed changes in aged HSC programs linked to specific accumulation of self-renewal and interferon primed old HSC clusters.**

PLZF, also known as Zbtb16, is a master transcriptional and epigenetic regulator with effects on growth, self-renewal and differentiation of hematopoietic cells. Our laboratory revealed an unexpected role of PLZF in restricting HSC aging by using a PLZF mutant mouse model ($Zbtb16^{lu/lu}$ mice). Besides, PLZF when fused to the **R**etinoic **A**cid **R**eceptor **α** (RARA) leads to **A**cute **P**romyelocytic **L**eukemia (APL with PLZF/RARA oncogenic fusion), an aging-related haematological malignancy. One of the principal objectives of the IMSUT project is to identify PLZF regulatory network involved in aging and aging-related diseases. For this purpose we also generated 12000 RNAseq from either young or old $Zbtb16^{lu/lu}$ HSCs. **We revealed cell cycle and metabolism defects on specific HSCs sub-populations upon PLZF deletion.**

To further decipher the transcriptional and epigenetic events related to PLZF activity and involved in

hematopoietic aging and aging-related diseases, Iwama's laboratory set-up single cell epigenomic analyses (single-cell ATAC-seq) on purified APL (PLZF/RARA) murine samples. In parallel, we performed single-cell transcriptomic analyses on this APL and did the bioinformatics integration of epigenomic and transcriptomic datasets. **We revealed that APL onset, maintenance and its resistance towards therapeutics involve the epigenetic activity of Enhancer of the Zest 2 (EZH2).**

Future objectives

Within the second year of the IMSUT project (2021-2022) we will complement our epigenomic and transcriptomic analyses by analyzing additional time points. Epigenomic and transcriptomic datasets will be integrated in order to identify key pathways involved in HSC aging. We aim also to validate the relevance of some of these pathways as potential intervene points.