P983 SINGLE CELL ANALYSIS ALLOWS THE EARLY DETECTION OF LEUKEMIC CLONES IN MPN PATIENTS

**Topic:** 15. Myeloproliferative neoplasms - Biology & Translational Research

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**Background:** Myeloproliferative neoplasms (MPNs) are a group of hematopoietic stem cell disorders resulting in the overproduction of myeloid differentiated cells. Primary Myelofibrosis (PMF) is characterized by the worst prognosis and 15-20% of cases develop secondary Acute Myeloid Leukemia (AML). MPNs driver mutations affect JAK2, CALR or MPL genes. Moreover, mutations in epigenetic regulators can exacerbate the disease and alter response to treatment. Our group recently demonstrated through single cell analysis that MPNs progression is due to increased genetic heterogeneity, loss of heterozygosity and parallel AML evolution.

**Aims:** In this work we sought to define the genomic architecture of MPN patients during disease evolution, and gain insight into the chromatin and transcriptional perturbations induced by epigenetic modifiers’ mutations.

**Methods:**

In order to describe MPNs clonal hierarchy at the single cell level, we employed Mission Bio Tapestry platform. We analyzed the CD34+ compartment of 3 patients during the chronic phase of the disease and after leukemic transformation through a custom panel comprising 29 genes frequently mutated in myeloid neoplasms. CNV assessment was performed on the same data through Mosaic algorithm.

On one patient, single nucleus RNA (snRNA-seq) and ATAC-seq were then conducted through 10x genomics instrument on CD34+ cells from the chronic and blast phase. Data were analyzed through Seurat R package.

**Results:**

Genomic analysis was performed on 20,652 single cells coming from 7 samples. The analyzed patients suffered from Essential Thrombocytopenia (n=2) or PMF (n=1); 2 patients harbored CALR type 1 driver mutation, while one patient carried JAK2V617F variant. In all patients, the first mutational hit occurred on epigenetic remodeler genes (i.e. TET2, ASXL1). Driver mutations’ allele frequency remained stable during disease progression and did not seem to drive leukemic transformation; in one case, JAK2V617F variant was lost in blast phase. In one patient, single cell analysis revealed the acquisition of 3 mutually exclusive pathogenic variants in RAS pathway (two NRAS mutations and a KRAS mutation). Notably, leukemic driver mutations, affecting genes such as IDH2, TP53 and KRAS, were already traceable at very low allele frequency in the chronic phase of the disease of all patients, despite being undetectable by bulk diagnostic NGS analysis. For all patients, CNV analysis highlighted a higher gene dosage imbalance in the leukemic clones when compared with those in the chronic phase.

Preliminary analysis of single cell ATAC+RNAseq data showed that the leukemic sample is enriched in more primitive cell types (e.g. multipotent progenitors). Moreover, genes found to be upregulated and more accessible in blast phase erythroid progenitors and HSC clusters are associated with AML poor prognosis.

**Summary/Conclusion:** Altogether this analysis suggests that MPNs’ first mutational hit frequently occurs in chromatin remodeler genes and affects a large fraction of neoplastic cells, confirming their impact on MPNs pathogenesis. Single cell multiomic analysis suggests that epigenetic alterations contribute to alter CD34+ cells differentiation state.
and activate the expression of pro-leukemic genes. Moreover, genomic analysis highlighted that clones carrying driver mutations remain stable during time and do not seem to drive leukemic transformation. On the other hand, genetic alterations, such as SNVs and CNVs, driving AML evolution are early identified by single cell analysis despite being undetectable by bulk sequencing.