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P328 DYNAMIC EVOLUTION OF TCF3-PBX1 LEUKEMIAS AT THE SINGLE-CELL LEVEL UNDER CHEMOTHERAPY PRESSURE

Topic: 01. Acute lymphoblastic leukemia - Biology & Translational Research

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. While research has been focused on high-risk patients, the biology of low-to-intermediate-risk patients has so far been inadequately investigated and can lead to overtreatment with severe side effects and under treatment with risk of relapse. The translocation t(1;19) codes for chimeric fusion protein TCF3-PBX1, which is associated with intermediate risk ALL. Using our previous generated TCF3-PBX1 conditional knock-in mice, we established a model to study in vivo chemotherapy resistance.

Aims: We hypothesize that chemotherapy and microenvironment play a crucial role in development of resistance in TCF3-PBX1 leukemias by influencing transcriptional regulation, activation of signaling pathways and the hierarchical structure of leukemic cells. In this project we aim to characterize dynamic changes of TCF3-PBX1 leukemia cells in different tissues under chemotherapy pressure.

Methods: Recipient C57/BL6 healthy mice were sub-lethally irradiated, transplanted with mouse TCF3-PBX1 leukemia cells and treated with vehicle (n=15), prednisolone (n=13) and daunorubicin (n=15) for 20 days. Mice were monitored for signs of disease regularly and circulating GFP+ TCF3-PBX1 leukemia cells were assessed by flow cytometry. Sick mice were sacrificed, leukemia cells were isolated from five different organs (bone marrow, spleen, lymph nodes, spinal cord and brain) and characterized by immunophenotyping, sanger sequencing, bulk RNA sequencing (bulk RNAseq) of GFP+ sorted cells, single cell RNA sequencing and mass cytometry (CYTOF).

Results: All mice transplanted with TCF3-PBX1 leukemia cells and treated with vehicle succumbed to disease with a median survival of about 70 days. We optimized chemotherapy drug concentration and transplanted cell dose, so 60% of mice treated with prednisolone or daunorubicin survived at least 150 days. Leukemic infiltration was showed by histological stainings in analyzed tissues including central nervous system (CNS) (spinal cord, brain) and GFP+ leukemia cells were quantified by flow cytometry. No major differences were observed in immunophenotype of TCF3-PBX1 leukemia and variant allele frequency (VAF) of the known PTPN11 mutation in analyzed tissues or depending on in vivo treatments. Bulk RNAseq of FACS-sorted GFP+ TCF3-PBX1 leukemia cells were clustered and CNS cells separated from other tissues. scRNAseq revealed additional heterogeneity within each tissue based on signaling pathway and cell cycle activity. Interleukin signaling, regulation of apoptosis pathways, and signaling by the B cell receptor (BCR) were regulated based on pathway analysis in CNS compared to other tissues. Hence, we elucidated the hierarchical structure of hematopoietic cells, interaction of leukemic cells with the microenvironment, and changes in signaling response to chemotherapy in vivo treatment by mass spectrometry (CyTOF) to validate the identified altered signaling pathway activities at protein level.

Summary/Conclusion: We have developed a mouse model in order to characterize in vivo chemotherapy resistance

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depending on niche. Global transcriptomics and phospho-proteomics at the single-cell level might elucidate novel mechanisms of chemotherapy resistance suitable for pharmacological therapies.

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