Abstract: P489

Title: CSF1R MARKS A SUBSET OF FETAL LMPP WITH ACUTE MYELOID LEUKEMIA PROPAGATION PROPERTIES IN A MLL-AF9 MOUSE MODEL

Abstract Type: Poster Presentation

Topic: Acute myeloid leukemia - Biology & translational research

Background:

The MLL-AF9 t(9;11) translocation, in infants, accounts for 17% of cases with acute lymphoblastic leukemia (ALL) and 22% of cases with acute myeloid leukemia (AML). The biology of MLL-AF9+ infant leukemia is poorly understood, particularly the mechanisms that drive the choice between the myeloid or the lymphoid lineage. Lymphoid-primed multipotent progenitors (LMPPs) are shown to possess both myeloid and lymphoid lineage potential, notably those that express CSF1R, as recently shown in the murine fetal liver context. Furthermore, CSF1R has been recently recognized as a therapeutic target in the treatment of AML.

Aims:

We aimed to uncover (1) whether the lineage choice in MLL-AF9+ ALL or AML could originate in these LMPPs expressing or not CSF1R, and (2) to evaluate the contribution of CSF1R in the initiation of MLL-AF9+ leukemia.

Methods:

Doxycycline-inducible MLL-AF9+ mice were obtained from Juerg Schwaller's lab, and MLL-AF9 expression was induced from embryonic day (E) 12.5 in pregnant females by administering DOX in the drinking water. E14.5 embryos were harvested and CSF1R+/- LMPPs sorted from MLL-AF9+ fetal livers. Their lymphoid and myeloid potential was assessed in vitro by performing colony-forming (CFU-C) and co-culture assays, *in vivo* through transplants into NSG mice, and transcriptomically by bulk-RNA-sequencing

Results:

Our results show that MLL-AF9-expressing CSF1R+ LMPPs are more proliferative and plastic than CSF1R- LMPPs, since they produced more blast-like colonies *in vitro,* with a mixed lymphoid/myeloid phenotype under myeloid conditions. *In vivo*, we observed a lymphoid-biased donor reconstitution (B220+CD19+CD43+) in CSF1R- LMPPs recipients, although this switched to myeloid-associated disease when they got sick. CSF1R+ LMPPs recipients had a myeloid-biased reconstitution from the beginning and a shorter disease latency compared with CSF1R- cells. Only CSF1R+ cells from primary recipients could propagate the disease in secondary recipients, with an AML phenotype. Our transcriptomic data indicate that CSF1R+ LMPPs possess a stem-like/HSC self-renewal-associated signature, with hematopoietic/leukemic stem cell genes (i.e. Mllt3, Socs2, Kit, Mecom, Erg, Gata2), AML-associated genes (i.e. Arhgap6, Cd79a, Ikzf2, Armcx1, Menin1, Meis1, Kdm5b) being upregulated. GSEA showed enriched terms for AML. Comparative analysis of our dataset with published sc-RNA sequencing datasets of pediatric AML patients (Zhang Y. et al. Genome Biol 24, 199; 2023) revealed our CSF1R+ LMPPs resemble HSC-like/leukemic stem cells (LSCs), as we observed expression of Mllt3, Erg, Gata2, and Hopx genes, and downregulation of OXPHOS (Slc25a1, Cdk1, Timm13, Timm21, Mrpl12, Mrps1, Mki67) genes in our CSF1R+ LMPPs and pediatric AML patients. Chemical inhibition of CSF1R (with GW2580) reduced the percentage of CD11b+ myeloid cells produced by CSF1R+ LMPPs at the end of the culture, compared with CSF1R+ LMPPs alone, which retained their mixed phenotype (B220+CD11b+). This indicates that CSF1R+ is not just a population marker, but also performs a functional role.

Summary/Conclusion:

Our data highlight the existence of a murine fetal population (CSF1R+ LMPPs) with leukemia-initiating cell and leukemic stem cell properties in the context of MLL-AF9+ AML. MLL-AF9+ CS1FR+ LMPPs share gene expression patterns with HSC-like/LSC cells identified in pediatric patients, with LSC genes being highly

expressed and OXPHOS downregulated. Functional validation of downstream signaling pathways of CSF1R is in progress to understand their involvement in the observed lineage choices.

Keywords: Mouse model, 11q23, Hematopoietic stem and progenitor cells, Acute myeloid leukemia