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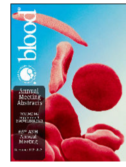

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Deciphering the Role of RAS Pathway Mutations in the Biology of Human Acute Myeloid Leukemia Using In Vivo Models

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Acute myeloid leukemia (AML) is a genetically heterogeneous hematologic malignancy for which the mutational profile of the leukemic cells is the most important prognostic factor for disease-free survival and overall survival. The failure of chemotherapy to completely eradicate the leukemic clones is the most common cause of death in AML. The genetic events that can contribute to chemoresistance are therefore important factors for the clinical outcome, but the relative importance of these events on chemoresistance and persistence of leukemia stem cells (LSC) remains poorly defined. MLL rearranged AMLs account for 5-10% of all AMLs and the RAS pathway is the most frequently mutated pathway in this sub-group of AMLs. The goal of this project is to study the role and biology of RAS pathway mutations in clonal evolution in human MLL-rearranged AML.

Methods

We have previously shown that human cord blood CD34+ cells transduced with a retrovirus encoding the MLL-AF9 fusion gene with an EGFP maker generate human AML when injected into immunodeficient NSG-SGM3 mice. Using a similar experimental design, we added a second hit to the CD34 transduced cells before their injection into NSG-SGM3 mice. MLL-AF9 cells were electroporated with Cas9 enzyme for the control and with Cas9 enzyme plus a single guide RNA (sgRNA) designed to induce the KRAS G13D or the NRAS G12D mutation. Mice were sacrificed when they showed sign of disease and the bone marrow and spleen were collected and analyzed. Kaplan-Meier curves, flow cytometry analyses, RNA and exome sequencing and secondary transplantation were performed on the samples.

Results

Mice injected with MLL-AF9 + KRAS G13D got sick around day 80 after injection and around day 60 for MLL-AF9 NRAS G12D, whereas it took ~150 days for control mice (MLL-AF9 KRAS WT) to get sick (fig 1). In multiple experiment, the latency was roughly cut by half by the KRAS G13D and NRAS G12D mutations. The cell surface phenotype and morphology were not significantly different between MLL-AF9 WT and MLL-AF9 KRAS/NRAS mutated. The variant allele frequency (VAF) for the KRAS mutation invariably increased from the day of injection to the day of sacrifice, where the percentages varied between 20-40%, which is typically observed in AML patients. In contrast, the VAF for NRAS mutated cells was lower (3-9%) but seems to be very aggressive into mice.

We performed limiting dilution analyses in secondary mice to quantify the LSC frequencies in MLL-AF9 WT and KRAS G13D / NRAS G12D leukemia. The average frequencies of control MLL-AF9 AMLs were between 1/510 000 to 1/1 450 000, while MLL-AF9+KRAS G13D AMLs frequencies were >1/45 000 and 1/7 800 for MLL-AF9+NRAS G12D AMLs, demonstrating that these 2 mutations increase the LSC frequency by >20-100 fold in our modelled MLL-AF9 AML.

Using transcriptome analyses, we looked at the gene expression changes induced by the KRAS G13D mutation in comparison to MLL-AF9 leukemias. These experiments are in progress for the NRAS G13D mutation. Many more genes were overexpressed in MLL-AF9 KRAS G13D vs MLL-AF9 KRAS WT than down regulated. Genes from the hedgehog (Hh) pathway such as Patched1 (PTCH1) and Smoothened (SMO) were amongst the genes the most differentially overexpressed by the KRAS mutation. It is particularly interesting because they have been shown important in stem cell biology and could explain the

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increase in LSC. Some SMO inhibitors have been tested in clinical trial and Glasdegib is FDA approved in combination with low dose cytarabine in elderly patients. Its effect on MLL leukemias or on KRAS mutated AMLs has not been tested/reported in a clinical study. Experiments are currently in progress to test the effect of Glasgebib on engraftment of MLL-AF9 KRAS mutated cells in our experimental setting.
Single cell RNA sequencing data on the three conditions will be presented at the meeting.
Given the success of generating these two point mutations, we are currently generating more RAS pathway mutations, including other KRAS mutations, PNTP11 and NF1 mutations.

Conclusion

These experiments showed: 1) It is possible to induce 2 oncogenic hits in human primary cells and get leukemia in vivo; 2) the KRAS G13D and NRAS G12D mutations shorten the latency of the disease and 3) increase the LSC frequency in secondary mice; 4) a possible involvement of the Hh pathway on stemness/LSC in RAS mutated cells; 5) our experimental approach is robust and very promising to decipher the RAS pathway in human MLL leukemias.

Disclosures No relevant conflicts of interest to declare.

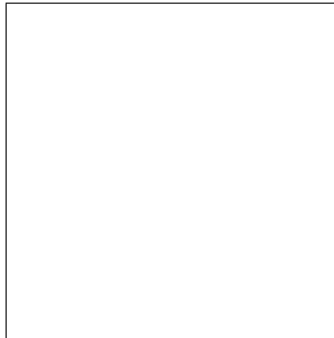

Figure 1

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