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ORAL ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Platelet Factor 4 Binds to Low-Density Lipoprotein Receptor to Block Leukemic Stem Cell Expansion and Recurrence

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Healthy hematopoietic stem cells (HSCs) reside in highly specialized microenvironments within the bone marrow, commonly referred to as niches. HSC activity is tightly regulated by various cellular and molecular niche constituents that ensure healthy hematopoiesis. However, through the accumulation of mutations, hematopoietic stem and progenitor cells can give rise to leukemia-initiating cells or leukemic stem cells (LSCs), which play a vital role in the onset, progression, and treatment outcome of acute myelogenous leukemia (AML). In AML, the expansion of the leukemic clone is associated with impaired healthy hematopoiesis, resulting in severe anemia, thrombocytopenia, and immunodeficiency, which can lead to severe morbidity. Current therapeutic options mainly eliminate the bulk of leukemic cells but do not efficiently target LSCs, leading to the high rates of relapse seen in AML patients. Recent studies suggest that LSCs hijack and alter the healthy bone marrow niche to better support their maintenance and proliferation. Understanding how LSCs interact with the bone marrow microenvironment is crucial for eradicating LSCs and preventing AML relapse.

Our previous work showed that platelet factor 4 (PF4), a chemokine stored in platelet alpha-granules and secreted by bone marrow megakaryocytes, can induce quiescence in healthy myeloid-biased HSCs (*Nature Medicine* 2014; *Developmental Cell* 2018). Here, we investigate the regulation of malignant LSCs by PF4 using mouse and human AML models. In an aggressive mouse AML model, MLL-AF9, our results show that recombinant PF4 blocks the proliferation of phenotypic LSCs (Lineage $^{-}$ IL7Rα^{-} Sca1 $^{-}$ MLL-AF9-GFP $^{+}$ c-Kit ^{high} CD34 $^{+}$ FcγRII/III ^{high}) *in vitro* (70% reduction; *P*=0.0279). Strikingly, cell cycle analyses revealed that *in vivo*, both LSCs and bulk AML proliferation are significantly inhibited 24 hours post-PF4 (80 mg/kg) injection, resulting in a significantly lower leukemic burden. By contrast, *Pf4* $^{-/-}$ mice exhibited rapid AML disease progression via increased LSC proliferation. Furthermore, using a genetic mouse model (*Pf4-Cre; iDTR*) where PF4-secreting megakaryocyte cells can be depleted *in vivo* in leukemic mice, our results revealed that megakaryocyte depletion led to poor AML survival due to accelerated disease progression (Log-rank *P*<0.0017). These results demonstrate that megakaryocyte-derived PF4 can directly regulate the proliferation of leukemia cells. Interestingly, our results indicate that AML drives striking alterations in bone marrow megakaryocytes, including a lower frequency, reduced cell maturation, and reduced secretion of PF4 (~80% reduction, P<0.0001) compared to healthy controls. Accordingly, PF4 levels are reduced in the serum of myelodysplastic syndrome patients where hyperproliferative malignant cells are found alongside dysplastic megakaryocytes.

To assess whether recombinant PF4 can alter the course of AML relapse in a preclinical experimental setting, we treated moribund leukemic mice with the chemotherapeutic drug Cytarabine followed by 3 daily I.V. injections of PF4 (80 mg/kg) or saline. Strikingly, this short treatment with PF4 preferentially reduced the frequency of LSCs *in vivo* during the relapse phase of AML (75% reduction; *P*=0.05). Furthermore, mice transplanted with MLL-AF9 AML cells that were pre-treated with Cytarabine and PF4 *in vitro* had prolonged overall survival (Log-rank *P<0.0132*) compared to Cytarabine alone. Importantly, two of six mice had no detectable AML up to 150 days post-transplantation. Finally, single-cell RNA sequencing experiments revealed that PF4 signals through Low-Density Lipoprotein Receptor (LDLR) on LSCs, whose expression is significantly upregulated upon Cytarabine treatment, during the relapse phase. Functional studies with fluorescently labeled low-density lipoprotein (LDL) confirmed that PF4 limits LSC uptake of LDL via LDLR to block LSC proliferation (*P=0.0044*), suggesting an important role for PF4 in AML metabolism. Altogether, our studies reveal an unknown function for PF4 in AML and highlight PF4's potential as an effective adjuvant therapy in preventing AML relapse by inhibiting the recurrence of chemotherapeutic-resistant LSCs.

Disclosures No relevant conflicts of interest to declare.

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