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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

H2-K1 on *MLL-AF9* Leukemia Cells Facilitates the Escape of NK Cell-Mediated Immune Surveillance

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Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults, and prognosis is poor; 5-year survival approximately 30%. Recently, there is an emerging recognition of the innate immune system, in particular Natural killer (NK) cells and macrophages, for immune surveillance against AML, but the mechanistic basis for this is mostly unknow. Identifying how AML cells escape immune surveillance by NK cells may translate into new treatment opportunities for AML patients.
To identify new therapeutic opportunities, we recently performed an *in vivo* CRISPR/Cas9 screen targeting 961 cell surface genes using the *MLL-AF9* AML mouse model. One of the top leukemia stem cell dependencies in the bone marrow niche was *H2-K1*, an ortholog of human *HLA-A*, a classical MHC class-I molecule. In validation experiments, we observed a seven-fold depletion ($p<0.001$) of *H2-K1* sgRNA-expressing c-Kit $^{+}$Cas9 $^{+}$ leukemia cells in the bone marrow. In contrast, genetic disruption of *H2-K1* did not impact the growth and survival of *MLL-AF9* leukemia cells in culture. Given the known suppressive role of MHC class-I molecules for immune cells, we speculated that *H2-K1* mayprovide inhibitory signals that counteract immune-surveillance mechanisms in the bone marrow niche. To test this hypothesis, we depleted NK cells and macrophages prior to transplantation of the c-Kit $^{+}$ *MLL-AF9* leukemia cells. Macrophage depletion by clodronate liposomes did not affect the *in vivo* expansion of *H2-K1* sgRNA-expressing leukemia cells demonstrating that macrophages were not suppressed by *H2-K1* on the leukemia cells. In contrast, NK1.1 antibody-mediated depletion of NK cells fully rescued the depletion of *H2-K1* sgRNA-expressing leukemia cells *in vivo*. These findings suggest that *H2-K1* expression on *MLL-AF9* leukemia cells inhibits NK cells in this model. Consistent with these findings, *H2-K1* knockdown in leukemia cells triggered a two-fold increase of INFγ production ($p<0.01$) in the NK cells, accompanied by augmented apoptosis of the leukemia cells ($p<0.01$).
Given that NK cells have been shown to be dysfunctional in AML patients, we next explored whether leukemia development affects NK cell maturation. The expansion of leukemia cells in the mice skewed NK cells towards a M1 (CD27 $^{+}$CD11b $^{-}$) state and decreased the level of the more cytotoxic M2 (CD27 $^{+}$CD11b $^{+}$) and M3 (CD27 $^{-}$CD11b $^{+}$) populations. *H2-K1* disruption in the leukemic cells restored the level of M2 and M3 NK cell population in the bone marrow. Notably, restoration of matured NK cell populations was accompanied by an increased expression of NKG2D, an activating receptor, indicating a more cytotoxic state of the NK cells. In line with these findings, ablation of *H2-K1* in leukemia cells induced JAK/STAT and NF-$\kappa\beta$ signaling in the NK cells.
Taken together, our study identifies that *H2-K1* on *MLL-AF9* leukemia stem cells facilitates immune evasion by suppressing NK cells. *H2-K1* alters the maturation and activation of NK cells in the bone marrow niche. These findings increase our understanding of how leukemia cells escape immune surveillance and suggest that the identification of corresponding mechanisms in human AML could pave the way for new therapies that boost the endogenous NK cells by restoring immune surveillance mechanisms.

Disclosures No relevant conflicts of interest to declare.

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