S116 CELLULAR AND MOLECULAR MECHANISMS OF EVI1-EXPRESSING MLL-REARRANGED ACUTE MYELOID LEUKEMIA

Topic: 03. Acute myeloid leukemia - Biology & Translational Research

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Background:

Expression of a doxycycline (DOX)-inducible acute myeloid leukaemia (AML)-associated iMLL-AF9 fusion transgene in long-term haematopoietic stem cells (LT-HSC) can lead to an invasive and chemo-resistant disease expressing the transcription factor EVI1. High EVI1 expression has been suggested as marker of poor outcome in AML patients even without rearrangements of the EVI1 locus at 3q26.

Aims:

We addressed the association between EVI1 expression, the cellular origin and poor disease outcome in AML driven by the iMLL-AF9 fusion gene.

Methods:

The role of EVI1 expression was studied in iMLL-AF9 transgenic mice carrying an Evi1-IRES GFP reporter in vitro using flow cytometry, colony formation and RT-qPCR assays, ex vivo with high-resolution bone marrow (BM) imaging, and in vivo, by transplantation of enriched naïve Evi1+ iMLL-AF9 hematopoietic stem and progenitor cells (HSPC) into irradiated recipients on DOX. Haematopoiesis of symptomatic mice was analysed by flow cytometry and histology. For mechanistic studies, single cell and bulk RNA sequencing was performed on enriched HSPC or BM samples from diseased mice.

Results:

Analysis of BM cells from Evi1-IRES-GFP reporter mice revealed that not only the mostly quiescent LT-HSC but also fractions of the more proliferating multipotent progenitors (MPP1-3) express abundant Evi1 (“Evi1\text{high}”). Induction of the iMLL-AF9 fusion did not result in significant changes in numbers of Evi1+ cells nor levels of Evi1 mRNA expression in the LT-HSC and MPP1 compartments. However, in colony assays, Evi1\text{high} iMLL-AF9 cells retained a more immature phenotype and produced more colonies with an invasive morphology than Evi1\text{low} cells (n=11, p<0.05). While Evi1 expression did not influence disease induction upon transplantation of LT-HSC, recipients of Evi1\text{+} MPP1 cells developed AML earlier than Evi1\text{−} MPP1 (n=11, 79 vs. 269d, p<0.05). Disease induced by Evi1+ cells presented with more extensive leukemic organ infiltration than Evi1+ AML. Evi1 expression also correlated with in vitro Ara-C resistance. We also examined whether some exogenous factors may increase AML susceptibility by expanding the Evi1+ HSPC. Although a single injection of recombinant mouse thrombopoietin (TPO) only increased the number of LT-HSC and not of MPP1, the Evi1\text{high} cell fraction was enlarged in both compartments (LT-HSC: 23 vs. 50%; MPP1: 22 vs. 47%; n=29, p<0.0001) supported by high-resolution imaging. Interestingly, increased TPO-induced HSPC cycling was confined to the Evi1\text{high} cell population (n=3, p<0.05). Transplantation of TPO-treated...
iMLL-AF9 LT-HSC or MPP1 resulted in a significantly faster induction of Evi1+ AML than controls (n=19, MPP1: 35 vs. 79d, p<0.001; LT-HSC: 41 vs. 90d, p<0.001). To better understand mechanisms of aggravated AML after TPO mediated expansion of Evi1+ HSCP we performed multiplexed single cell RNA sequencing of highly enriched HSCP cells in iMLL-AF9 and control mice. While we observed no changes of cellular cluster organisation 2 days after TPO injection, we found some differentially expressed genes in TPO-stimulated cycling iMLL-AF9 HSCP, including potential stemness regulators.

**Summary/Conclusion:**

Our results suggest that expansion of Evi1-expressing HSCP by exogenous factors can result in a more aggressive MLL-AF9-driven AML. Ongoing data exploration and validation may characterize aberrantly expressed genes in TPO-stimulated Evi1+ iMLL-AF9-expressing HSCP as potential therapeutic targets to impair stemness of AML cells.