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P456 LEUKEMOGENESIS BY AML-SPECIFIC DRIVER MUTATIONS WITHIN DNMT3A, IDH2 AND NPM1

Topic: 3. Acute myeloid leukemia - Biology & Translational Research

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Background:

Acute myeloid leukemia (AML) develops from somatic driver mutations. A group of non-random point mutations in epigenetic modifiers (EMs), DNMT3A, TET2/IDH1 or 2, in combination with NPM1mut, showed a strict functional association, strongly suggesting a crucial role for the balance between methylation and demethylation in the pathogenesis of AML. Methylation is a dynamic process related to oxidative demethylation whose intermediates represent DNA damage signals with the potential to increase mutagenesis and transformation if not accurately repaired. This is also relevant for those with DNMT3A mutations linked to clonal hematopoiesis and an increased risk of AML.

Aims:

This study aimed to clarify the role of each mutated factor and their combination for determining the leukemic phenotype represented by increased self-renewal and differentiation block of leukemic blasts. In addition, we wanted to disclose whether leukemogenesis was related to aberrant methylation/demethylation. Therefore, we compared the effects of mutated EMs and NPM1 with their normal counterparts regarding their contribution to the leukemic phenotype.

Methods:

We retrovirally expressed the following constructs in murine Sca1+/lin- hematopoietic progenitor and stem cells (HPSCs): DNMT3A, DNMT3A^{R882H}, DNMT3A-IDH2, DNMT3A^{R882H}-IDH2^{R140Q}, DNMT3A-IDH2-NPM1, and DNMT3A^{R882H}-IDH2^{R140Q}-NPM1mutA. PML/RARα and DEK/NUP214 served as controls. Intracellular flow cytometry was used to assess the rate of 5'mC (methylation), 5'hmC, and 5'fC (demethylation). Surface marker expression (c-Kit, Sca1, Gr1, and Mac1) in the absence or presence of G/GM-CSF was used to investigate differentiation. Self-renewal and leukemogenic potential were studied in vitro by detecting serial replating efficiency and *in vivo* by colony-forming unit-spleen day 12 (CFU-S12).

Results:

The expression of DNMT3A led to hypermethylation and an increased rate of demethylation. The presence of the R882H mutation in DNMT3A reversed this. All other combinations of unmutated factors did not change the methylation/demethylation profile induced by DNMT3A alone. A similar effect was found when DNMT3A^{R882H} was combined with IDH2^{R140Q} and NPM1mutA, as they did not change the decreased methylation/demethylation levels induced by DNMT3A^{R882H}.

Only DNMT3A^{R882H} exhibited reduced myeloid differentiation, as shown by an increased c-Kit expression compared to DNMT3A. The addition of G/GM-CSF abolished this differentiation block.

DNMT3A^{R882H}, DNMT3A^{R882H}-IDH2^{R140Q}, and DNMT3A^{R882H}-IDH2^{R140Q}-NPM1mutA increased serial replating suggesting an increased self-renewal. The in vitro data were confirmed by our *in vivo* findings in the CFU-S12. This was mainly due to DNMT3A^{R882H}, as all combinations with DNMT3A^{R882H} resulted in an increased colony number indicating an increased stem cell capacity and leukemogenic potential.

Summary/Conclusion:

Mutations in DNMT3A can reduce DNMT3A methylating activity, resulting in lower DNA methylation levels and, therefore, an altered epigenetic regulation. Our data shows that lower DNA methylation levels contributed to leukemogenesis by inducing aberrant self-renewal and a differentiation block in HPSCs.