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S122 MUTANT IDH1 HIJACKS DIFFERENTIATION PROGRAMS IN MYELOID PROGENITOR CELLS TO IMPAIR GRANULOCYTIC DIFFERENTIATION.

Topic: AML biology and clinical prediction

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Background:

Isocitrate dehydrogenase 1 (*IDH1*) mutations are common genetic lesions in acute myeloid leukemia (AML) and are considered as early events during pathogenesis despite rarely being detected in clonal hematopoiesis. Clinically relevant mutations in *IDH1* are always heterozygous and result in neomorphic enzymatic activity leading to the production of the oncometabolite (R)-2-hydroxyglutarate which has been associated with DNA and histone hypermethylation. However, the intricate molecular alterations resulting from *IDH1* mutations and how they contribute to malignant transformation are only poorly understood.

Aims:

The aims of this study were to investigate the cellular and molecular aberrations elicited by mutant *Idh1* in hematopoiesis in order to better understand the mechanisms driving *IDH1*-mutant (pre-)leukemias.

Methods:

We established an inducible mouse model expressing the *Idh1*-R132H mutation from the endogenous gene locus together with a YFP-reporter. Expression of a tamoxifen-inducible Cre was driven by the Scl enhancer (Scl-CreERT), resulting in activation of *Idh1*-R132H and YFP expression in hematopoietic stem and progenitor cells (HSPC). Lineage negative / YFP+ cells were transplanted into lethally irradiated mice. Blood and bone marrow cells from fully chimeric *Idh1*-WT and *Idh1*-R132H mice were used for flow cytometry analyses, scRNA-sequencing (10X platform), and *ex vivo/in vivo* functional assays.

Results:

Analysis of scRNA-seq data of normal human and murine bone marrow revealed dynamic regulation of *IDH1/Idh1* expression in hematopoiesis with the highest expression in myeloid progenitor (MP) cells. Two transcript variants (TV) of *Idh1* encoding for the same protein are found in the murine genome. Specific upregulation of *Idh1*-TV2 was

observed in MPs isolated from C57BL/6 mice. In *Idh1*-R132H (*Idh1*-mut) mice, this upregulation strongly correlated with a significant expansion of MPs ($^{CD55-}$CMPs, $^{Ly6C+}$GMPs), a significant loss of granulocytes and a significant increase in Ly6C^{high} monocytes. Adoptive transfer experiments confirmed that *Idh1*-mut $^{CD55-}$CMPs produce less granulocytes than the WT controls, suggesting that *Idh1*-mut MPs exhibit a cell-intrinsic defect in granulopoiesis. Treatment of *Idh1*-mut $^{CD55-}$CMPs with a mutant-specific inhibitor significantly reduced the aberrant production of Ly6C^{high} monocytes in an *ex vivo* differentiation assay. Further, scRNA-seq revealed that late neutrophil progenitors are depleted in *Idh1*-mut mice and show reduced expression of the granulocyte transcription factor *Cebpe*. To test the relevance of our findings for AML patients, we compared DNA methylation data from *IDH1*-mut AML samples with data from normal human hematopoietic cell types. This revealed epigenetic scars indicating granulocytic lineage commitment in 9/16 *IDH1*-mut samples, while none of the *IDH2*-mut samples (n=29) tested revealed such scars. This suggested that granulocytic differentiation defects might contribute to leukemogenesis in *IDH1*-mut AML.

Summary/Conclusion: In the present study, we identified cellular defects in the myeloid progenitor cell compartment of *Idh1*-mut mice. These effects were mediated by a physiologic upregulation of *Idh1* expression, which resulted in downregulation of *Cebpe* in neutrophil progenitors and impaired granulopoiesis. Identification of granulocytic epigenetic scars in *IDH1*-mut AML samples suggest granulocytic differentiation defect might also play a role in leukemogenesis in humans. In summary, for the first time, we present molecular and cellular data that explain the association of *IDH1* mutations with myeloid neoplasms.