ELSEVIER

Blood 142 (2023) 4063

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

Asxl1-mutant Dominant Clones Have an Erythropoietic Lineage Deficiency through a Disturbed Epigenetic Landscape

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Two hematologic features of red blood cells (RBCs) rank among the top three high risk factors associated with increased risk of malignant progression from clonal hematopoiesis (CH). These risk factors are macrocytosis as measured by elevated mean corpuscular volume (MCV) of 100 fL or greater and red cell distribution width (RDW) of 15% or greater. It is not known how these changes in RBC parameters are related to the leukemic transformation. Without major disturbance in the quantitative output of RBCs, these changes may reflect clone specific changes in erythropoiesis that are averaged in such measurements. We used an *in vivo* model of CH in zebrafish to study epigenetic and transcriptional changes in mutant clones, focusing on the erythropoietic program. In this model, mosaic somatic mutations in exon 12 of *asxl1* with CRISPR-Cas9 mutagenesis are combined with color labeling of hematopoietic stem and progenitor cell (HSPC) clones. These mosaic *asxl1* mutants exhibit an expansion of single-colored mutant clones to greater than 30% of hematopoietic output. No significant hematopoietic disturbance has been noted in these mutants by flow cytometric and morphologic analysis. To study clone specific effects of this epigenetic factor mutation, we sorted HSPCs from the dominant *asxl1*-mutant clone and compared it to HSPCs from control animals using transposase-accessible chromatin with sequencing (ATAC-seq) assay. Among over 22,000 peaks, we identified 998 (4.5%) that increased and 938 (4.2%) that decreased by 2-fold in *asxl1*-mutant clones compared to controls. Of the upregulated peaks, 73% were distal elements (+/- 100kb from transcriptional start sites, TSS) and 27% were proximal or promoter peaks (-2kb to +500bp from TSS), while in decreased peaks 67% were in distal elements and 33% in the proximal/promoter region. Pathway analysis of assigned genes related to decreased peaks identified a striking loss of the erythropoietic program and the heme biosynthesis process. HOMER analysis of sequences within the decreased peaks showed significant association with GATA (q-value <0.001) and TRPS1 (q-value <0.001) motifs. This was confirmed by individual assessment of gene loci for lineage-specifying transcriptional factors, such as *tal1* and *gata1a* and genes in erythropoiesis, including *alas2, cahz, tmod4* and the hemoglobin locus with known enhancers in *nprl3* gene . Using an independently generated mutant zebrafish cohort, we performed bulk RNA-sequencing on sorted dominant clone and control HSPCs. Downregulated genes were consistent with a loss of erythroid differentiation program, with up to 16-fold downregulation of *hbba1, hbaa1, alas2* and *cahz* although less pronounced or no significant changes were noted in erythroid transcription factors (*tal1, gata1a, lmo2*) . Our data show that *asxl1*-mutant clones have an erythropoietic lineage defect with reduced epigenetic accessibility at lineage-specific loci with selective changes in gene expression. This ineffective erythropoiesis characterized by large MCV in mutant RBCs would increase the RDW due to the mixed contribution from mutant and wildtype clones in the peripheral blood. Our findings offer a biologic basis of the strong clinical association of abnormal RBC characteristics with higher risk CH for malignant transformation.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-178343