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POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Cellular and Molecular Analysis of Hematopoietic Stem and Progenitor Cells in Shwachman-Diamond Syndrome

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Shwachman-Diamond syndrome (SDS) is an inherited multi-organ disorder with bone marrow failure (BMF), which may include neutropenia, anemia, and thrombocytopenia, as well as a high risk of myelodysplasia and leukemia. Hematological complications are common causes of morbidity and mortality in SDS. Around 90% of SDS patients have biallelic mutations in the *Shwachman-Bodian-Diamond Syndrome* (*SBDS*) gene, which plays an important role in ribosome biogenesis and hematopoietic cell survival. In a collaborative study led by Dr. John Dick (Science 2016) novel hematopoietic progenitor subsets were identified (noted as F1, F2 and F3), of which the abundance, clonal capacity, and function in disease states remain unknown. We hypothesized that cellular and molecular analysis of hematopoietic stem and progenitor cells (HSPC) including these novel progenitors would provide valuable insights into the pathogenesis of BMF in SDS.

Using **multiparametric flow cytometry** we quantified the following HSPC populations: hematopoietic stem cells (HSC), multipotent progenitors (MPP F1-F3), common myeloid progenitors (CMP F1-F3), megakaryocyte-erythroid progenitors (MEP F1-F3) and granulocyte-monocyte progenitor (GMP) in 13 SDS patients with *SBDS* mutations and 10 normal control donors. We found a significant reduction in CD34+/CD38- and CD34+/CD38+ cells in SDS patients. It is particularly noteworthy that the sub-populations of CMPs (F1 and F2), and MEPs (F2 and F3) were prominently decreased. These cells contribute to the production of myeloid, erythroid, and megakaryocytic cells with different proportions.

Using **single-cell colony-forming assays**, we assessed the cloning efficiency of each HSPC, revealing that HSCs, MPPs and GMPs from SDS patients exhibited a significantly lower cloning efficiency compared to those from control subjects. Notably, the typical erythroid-committing progenitor cells (CMP F2, CMP F3, MEP F2, and MEP F3) in SDS collectively produced approximately 46% fewer erythroid cells per colony compared to control progenitors, while myeloid-committing progenitor cells (CMP F1, MEP F1) from these patients produced approximately 86% fewer myeloid cells, including neutrophils, per colony compared to the corresponding control cells. Further, HSCs and MPPs-F1 cells from SDS patients had a reduced cloning efficiency of approximately 22% and 27%, respectively, compared to control HSCs and MPP-F1 progenitors. These data suggest that multiple HSPC populations from SDS patients are characterized by reduced cell numbers, functional impairments, and dysregulation compared to normal subjects.

Next, we performed **transcriptome analysis** of each HSPC by RNA sequencing. We revealed dysregulated pathways and genes with the potential to disrupt hematopoiesis and mediate BMF in SDS. Using KEGG pathway analysis, we found that downregulation of the ribosome pathway reached statistical significance in HSC, CMP F1 and MEP F3. Downregulation of the hematopoietic cell lineage pathway reached statistical significance in MEP F1-2 and MPP F1-2. Additionally, the TGF-beta signaling pathway was suppressed in CMP F1-3, MPP F1, and GMP, while the NF-kappa B signaling pathway, crucial for regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells, was also found to be decreased in GMP.

Interrogation of specific dysregulate genes, we found that the hematopoietic transcription factor, *ZFP36 (*ZFP36 ring finger protein) which involved in cellular response to cytokines and growth factors, was significantly downregulated in five sub-populations of SDS HSPCs, particularly noticeable in the four progenitors (CMP F1-3, MEP F2, and GMP) which produced substantially fewer colonies in SDS patients compared to controls. Additionally, we observed downregulation of *TGFB2* and upregulation of *NFKBIA* and *NFKBIB* may inhibit the NF-kappa B signaling pathway in SDS patients. These alterations have the potential to influence the hematopoietic cell lineage pathway, leading to BMF. Functional assays are ongoing and aim to assess the impact of these dysregulated genes on formation and behavior of HSPCs.

In summary, our data suggest that multiple SDS HSPC populations are characterized by reduced cell numbers and functional impairments. As well, we revealed dysregulated pathways and genes with the potential to disrupt hematopoiesis and mediate BMF in SDS.

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