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POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Targeting Nucleotide Biosynthesis Ameliorates Neutropenia in a Zebrafish Model of Dnajc21-Mutant Shwachman-Diamond Syndrome

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Germline mutations in the ribosome maturation factors, *SBDS*, *DNAJC21 and EFL1*, lead to Shwachman-Diamond syndrome (SDS), a type of inherited bone marrow failure syndrome characterized by neutropenia, exocrine pancreatic insufficiency and growth restriction. Further, SDS patients have a 36% increased cumulative risk of developing myelodysplastic syndrome (MDS) and/or acute myeloid leukemia (AML) by 30 years of age. Given the rarity of SDS, large numbers of primary human samples are not readily available for mechanistic studies, warranting the use of animal models. Mouse and zebrafish models of *SBDS* mutations exhibit early lethality. In contrast, no *in vivo* models of *DNAJC21* mutations have been published to date. We generated the first *in vivo* model of *dnajc21* loss using zebrafish (*Danio rerio*), given their highly conserved hematopoietic program and ease of genetic manipulation. Introduction of a homozygous deletion mutation in the zebrafish *dnajc21* gene using CRISPR-Cas9 genomic editing resulted in stunted body growth and neutropenia, recapitulating hallmark SDS patient phenotypes.

Whole-mount *in situ* hybridization for key hematopoietic lineage markers revealed reduced *l-plastin* $^{+}$mature leukocytes and *hbbe3* $^{+}$ mature erythrocytes in *dnajc21* mutant embryos at 48 hours post-fertilization (hpf). A concomitant accumulation of *myb* $^{+}$ hematopoietic stem and progenitor cells (HSPCs) both in the dorsal aorta (equivalent to aorta-gonad-mesonephros) and the caudal hematopoietic tissue (fetal liver equivalent) was observed. Treatment of embryos with zebrafish *granulocyte colony stimulating factor (gcsf)* mRNA resulted in a modest expansion of neutrophils in *dnajc21* mutant zebrafish compared to wildtype. Further, we observed reduced cell proliferation marked by phosphohistone-H3 (pH3) as well as low ATP:AMP levels measured by liquid chromatography/mass-spectrometry (LC/MS) in the *dnajc21* mutants, suggesting a poor bioenergetic profile. At 4 months of age, flow cytometry and histopathological analysis identified reduced mature myelomonocytes and mild erythroid dysplasia in the whole kidney marrow (human bone marrow equivalent) of mutant fish.

The link between *TP53* somatic mutations and MDS/AML has been established in *SBDS*-mutant SDS, but has not been explored for *DNAJC21*-mutant SDS. We hence crossed our *dnajc21* mutant zebrafish with a zebrafish line carrying a gain-of-function mutation (R217H) in the *tp53* gene. Compound mutant fish had improved neutrophil counts but importantly, this was accompanied by an expansion of myeloid progenitors and significant erythroid dysplasia. To understand the disease mechanisms underlying the neutropenia and hypoproliferation in SDS, we performed bulk RNA sequencing of whole zebrafish larvae at 48 hpf. Biological processes such as oxidative stress response, drug catabolism and pre-mRNA spliceosome assembly were downregulated; whereas, acyl-CoA and nucleotide metabolism were upregulated in the mutants. In parallel, we performed untargeted metabolomics by LC/MS to determine the metabolic changes induced by *dnajc21* loss. Upregulated pathways included urea cycle, amino acid and phenylacetate metabolism. Pyrimidine biosynthesis, including both the *de novo* and salvage pathways were downregulated in the mutants. Remarkably, exogenous supplementation with uridine or thymidine significantly improved neutrophil counts in the mutants.

Our findings suggest that in *dnajc21*-mutant SDS1) neutropenia is caused by a combination of impaired granulocyte differentiation and cell proliferation; 2) the kidney marrow features myelodysplasia-like changes and 3) nucleotide biosynthesis may be a previously unrecognized therapeutic strategy in SDS.

Disclosures Berman: *Oxford Immune Algorithimics:* Membership on an entity's Board of Directors or advisory committees.

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