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PLENARY ABSTRACTS

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Metabolic Programming of Hematopoietic Stem Cell Function By Prenatal Folate

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Hematopoietic stem cells (HSCs) are programmed by metabolic changes resulting in alterations to HSC function. Here, we discovered that varying prenatal folate status metabolically programs HSCs during development and affects adult HSC self-renewal and engraftment potential into adulthood. Folate status varies greatly in the global population based on nutritional intake, common genetic polymorphisms, and widespread supplementation. Folate-derived one-carbon metabolism (OCM) regulates cellular methylation, *de novo* nucleotide biosynthesis and mitochondrial metabolism, processes critical to HSC function and establishment. Despite immense therapeutic potential, there is limited understanding of mechanisms driving HSC developmental programming. We hypothesize that varying prenatal folate status affects risk for hematologic dysfunction by developmentally programming HSCs.

To test how prenatal folate modulated HSC function, female mice were assigned to one of three experimental diets to model population-wide folate consumption: 0mg/kg (deficient, **FD**), 2mg/kg (control, **FC**) and 8mg/kg (supplemented, **FS**). Profiling of fetal liver (FL) hematopoietic cells revealed that FD diet caused expansion of long term (LT)-HSCs that was propagated across the hematopoietic hierarchy, including myeloid and lymphoid progenitors and mature myeloid cells. In contrast, FS decreased FL cell output. We next determined if these changes were driven by metabolic activity. FD fetal HSPCs exhibited higher mitochondrial membrane potential and lower glycolytic activity, whereas FS fetal HSPCs displayed both higher glycolytic activity and oxygen consumption despite impaired production of downstream hematopoietic cells. Together, these data suggest that HSPC metabolic programming initiates during fetal development and is folate-dose dependent.

To determine if prenatal folate programs hematopoiesis into adulthood, we quantified bone marrow (BM) hematopoietic cells in adult offspring after weaning onto standard chow. Surprisingly, FD expanded lymphoid progenitors in adult offspring with no impact on mature lymphoid cell output. In contrast, FS diet expanded myeloid and lymphoid progenitors in the BM as well as downstream mature cells, including monocytes and B-cells. To determine if these effects were HSC cell-intrinsic, we performed competitive transplantation assays of LT-HSCs isolated from adult offspring. FD offspring HSCs showed worse engraftment by chimerism across all peripheral blood (PB) populations compared to FC. In comparison, FS offspring HSCs showed enhanced total cellular output by donor cells/uL across all PB mature lineages except platelets. Secondary whole BM transplantation revealed that FS HSCs retained enhanced total cellular output and superior engraftment compared to FC, whereas FD HSCs demonstrated complete exhaustion. These data indicate that adult HSC function is programmed by prenatal folate status.

To investigate molecular alterations driving metabolic programming of adult hematopoiesis by prenatal folate, we performed single-cell RNA sequencing (Scseq) and metabolomics on adult offspring HSPCs. Scseq analysis revealed most differential gene expression (DE) in HSC clusters, with less DE in progenitors. Re-clustering of all HSCs revealed distinct heterogeneity within this population, where both FD and FS HSCs were identified as distinct clusters compared to FC. Metabolomic profiling further suggested that FD HSCs exhibited signs of mitochondrial dysfunction through, 1) accumulating glucose and lactate suggesting reliance on glycolysis and, 2) accumulating succinate and glutamine suggesting complex II impairment and inad-

equate utilization of mitochondrial metabolism. Surprisingly, the same changes associated with mitochondrial dysfunction in FD HSCs persisted downstream into lymphoid-biased multipotent progenitors. In contrast, limited metabolic changes were observed in FS HSCs, suggesting alternative pathways for sustained programming. These changes indicate that FD and FS offspring have distinct metabolic programming. Together, our data suggest that prenatal folate status programs hematopoietic stem cell function into adulthood and is driven by alterations to metabolic activity. Ongoing studies examine how prenatal folate status affects the adult hematological and immune response to challenge.

Disclosures No relevant conflicts of interest to declare.

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