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ORAL ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Isocitrate Dehydrogenase 2 Mutation Allows Myeloid Differentiation but Impairs Bone Marrow Macrophage Polarization and Function Via Metabolic Dysregulation

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Isocitrate dehydrogenase 2 (IDH2) mutations, while less common than other mutations associated with myelodysplastic syndromes (MDS), are also found in clonal hematopoiesis and are associated with increased risk of transformation to leukemia. IDH2 mutations lead to metabolic dysfunction and DNA hypermethylation due to the accumulation of D-2-hydroxyglutarate (2HG), an oncometabolite derived from alpha-ketoglutarate (aKG), which disrupts hematopoiesis. However, the mechanisms that induce clonal progression and transformation remain incompletely understood. Furthermore, the intrinsic and extrinsic impact of IDH mutations on mature myeloid cell functions and the bone marrow microenvironment remains to be elucidated. Previous literature suggested that hypermethylation via this mutant leads to incomplete differentiation and failed mature myeloid cell generation.

Our lab previously demonstrated that efferocytic defects in bone marrow macrophages can skew hematopoietic stem and progenitor cells via increased inflammatory mediators. Based on these data, we hypothesized that IDH2 mutations prevent complete polarization down pro-efferocytic phenotypes in macrophages due to blocking of metabolic pathways required for mature macrophage function. Congenic CD45.1+ mice were transplanted with retrovirally infected hematopoietic stem cells containing human WT or R172K IDH2 mutant with an mCherry reporter and showed macrocytic anemia, thrombocytopenia, and elevated reticulocytes by 16 weeks post-transplant. In contrast to prior literature, IDH2 mutants gave rise to mature reporter positive monocytes, macrophages, and neutrophils in the bone marrow and peripheral blood. As expected, we observed myeloid skewed hematopoiesis, a trademark of MDS. When cultured *ex vivo*, there was loss of efferocytic capacity in IDH2 mutant bone marrow macrophages when fed apoptotic neutrophils.

We then generated a genetic murine model using the Vav1 Cre IDH2 ^{R140Q} floxed mutant. The genetic model allowed us to study disease progression in the absence of radiation induced stress, and to observe mature myeloid populations while clinically defined MDS was absent. We performed ATACseq and bulk RNAseq analysis on bone marrow macrophages isolated from 3-month-old and 6-month-old mice, which demonstrated a significant decrease in global chromosomal accessibility; however, this had limited impact on the transcriptional program. Based on existing literature, the majority of macrophages in the bone marrow are in a poised state and have not yet activated genes associated with pro-efferocytic/pro-resolution or pro-inflammatory phenotypes. Since aKG is accumulated in macrophages during polarization toward reparative and pro-efferocytic phenotypes and is necessary for metabolic reprogramming for oxidative phosphorylation, we hypothesized that, in the presence of IDH2 mutations, which drain aKG from the system, pro-efferocytic polarized macrophages would be metabolically stunned. To test this theory, we isolated bone marrow macrophages from IDH2 mutants and cultured them to generate pro-efferocytic polarized macrophages. We then tested their efferocytic potential and metabolic capacity by seahorse assay. Continual efferocytosis, a hallmark of pro-efferocytic macrophages, was impaired in IDH2 mutant macrophages as early as 2-3 months of age. Consistent with this finding, mito stress testing by seahorse demonstrated that IDH2 mutant macrophages had severely impaired maximal respiratory capacity compared to WT macrophages. Treatment with global hypomethylating

agent azacytidine or a cell permeable aKG analog were unable to restore respiratory capacity or efferocytic activity in the IDH2 mutant. These data strongly suggest that the efferocytic defect in IDH2 mutant macrophages is due to metabolic rather than epigenetic reprogramming.
In summary, we identify defects in efferocytic function and oxidative phosphorylation in IDH2 mutant macrophages. This represents a novel mechanism that could alter the bone marrow microenvironment and impact progression of MDS and leukemic transformation. Furthermore, our data suggest that the metabolic dysregulation induced by IDH mutations may play a greater role than epigenetic impairment in IDH2 mutant mature myeloid cells, which could explain why approaches targeting hypermethylation have shown limited efficacy in clinical settings.

Disclosures No relevant conflicts of interest to declare.

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