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ORAL ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

Inflammatory Memory Restrains Intestinal Stem Cell (ISC) Regeneration after Allogeneic Stem Cell Transplantation (SCT)

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Introduction: Intestinal stem cells (ISCs) are responsible for the remarkable ability to maintain intestinal epithelium homeostasis and regeneration throughout life. Inflammatory damage of ISCs underpins injury caused by graft-versus-host disease (GVHD), inflammatory bowel diseases (IBD) and immune check-point blocker mediated colitis. However, it remains unknown whether the ISCs that survive or tolerate inflammation are fully functional and can return to their full functionality after the resolution of ongoing inflammatory insults. Herein we investigated the consequences of inflammation from GVHD on Lgr5+ISCs in multiple well-defined clinically relevant models of gastro-intestinal acute graft-versus-host disease (GI GVHD).
Methods: We utilized single cell RNA (scRNA) sequencing to assess transcriptomics, Assay of transposase-accessible chromatin sequencing (ATAC-seq) to assess epigenomics, and functional metabolomics of ISCs in clinically relevant in vivo models of GVHD after major histocompatibility complex (MHC)-disparate BALB/c→C57BL/6 (B6) and MHC matched minor mismatched C3H.sw→B6 models of allogeneic SCT. *Ex vivo* intestinal organoids cultures, mitochondrial, and functional biochemical assays were utilized to determine the biological relevance of the changes observed from the 'omic' analyses and further validated them *in vivo* by developing novel Lgr5 $^{+}$ISC specific succinate dehydrogenase A (SDHA) knock-out mice.
Results: We examined the transcriptomes of Lgr5+ISCs with scRNA-seq of the intestinal crypts harvested from MHC-disparate BALB/c→B6 recipients on day +7 after allogeneic SCT. Bioinformatic analyses demonstrated upregulation of interferon and inflammation response genes but significant downregulation of genes involved in mitochondrial function, its complexes including complex II (SDH), ATP metabolic process, OXPHOS, and cytoplasmic translation. To assess the impact of metabolic functional gene changes, we harvested and assessed ISCs in *ex vivo* organoid cultures from transplanted recipients in the absence of ongoing inflammation. The ISCs harvested from GVHD animals demonstrated significantly reduced regeneration, differentiation and oxygen consumption rates (OCRs) with no change in extracellular acidification rates (ECAR) by Seahorse. FACs analysis confirmed reduction in SDHA component of mitochondrial complex II in Lgr5 $^{+}$ISCs, demonstrating mechanistic cause for reduction in OXPHOS in ISCs. We next generated and utilized Lgr5 $^{+}$ ISC-specific SDHA KO mice as GVHD recipients and found that demonstrated significantly greater mortality when compared to the WT littermate recipients ($P<0.01$). Biochemical analyses demonstrated increased levels of succinate, a metabolic intermediary with known epigenetic and inflammatory functions, in ISCs harvested from GVHD animals. We therefore hypothesized that the reduction in ability to form functional organoids *ex vivo*, in the absence of ongoing inflammation, by the ISCs harvested from GVHD hosts is because of the inflammation epigenetic reprogramming induced by succinate mediated changes in DNA methylation (5-mC) of the ISCs in GVHD recipients. Consistent with the hypothesis greater DNA methylation, with alteration of epigenome by ATAC-seq was observed in ISCs sorted from GVHD recipients. Integrative analyses demonstrated correlation between epigenomic changes and transcriptomic changes. Finally, we analyzed whether the inflammation epigenomic induced changes in the GVHD ISCs with serial *ex vivo* organoid cultures and *in vivo* by transfer into secondary hosts. Both *ex vivo* and *in vivo* studies demonstrated poor regeneration from GVHD ISCs demonstrating retention of maladaptive memory in ISCs following their exposure to inflammation during GVHD.
Conclusions: GI GVHD induced inflammation causes not only quantitative loss of ISCs but also induced qualitative changes in the surviving ISCs. Inflammation induced OXPHOS deficiency in Lgr5 $^{+}$ ISCs leads to accumulation of succinate that reprograms the epigenome and restrains their subsequent regeneration potential.

Disclosures No relevant conflicts of interest to declare.

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