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POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

The DPP4 Dependent Transcriptome Identifies Pathways to Improve Mouse Hematopoietic Cell Function

James Ropa, BS, MSc, PhD[1], *Scott Cooper, MS*[2], *Maegan L Capitano, PhD*[3], *Mark H Kaplan, PhD*[3]

[1]Department of Microbiology and Immunology, Indiana University School of Medicine, Avon, IN
[2]Indiana University School of Medicine, Indianapolis, IN
[3]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN

Hematopoietic cell transplantation (HCT) is a curative treatment for hematologic disorders wherein healthy donor hematopoietic stem (HSCs) and progenitor cells (HPCs) collected from bone marrow (BM), mobilized peripheral blood (PB) or umbilical cord blood (CB) reconstitute an ablated hematopoietic system. There is a strong need to enhance functional competency of HSCs/HPCs for HCT to prevent complications like graft versus host disease (GVHD), relapse, and graft failure. Dipeptidyl peptidase 4 (DPP4) is a protein that cleaves N-terminal dipeptides from hematopoietic factors, impacting their function. Inhibition of DPP4 improves HSC/HPC competency in mouse models of HCT and clinically speeds neutrophil recovery after CB transplantation and reduces incidences of GVHD after PB transplantation. Thus, DPP4 inhibition is a viable component of a multi-pronged strategy to improve engraftment and prevent GVHD.

While DPP4 inhibition improved early neutrophil recovery in clinical trials, the effects were modest, required high dose treatment, and effects on platelet recovery were unclear. Examining mechanisms by which DPP4 inhibition drives HSC/HPC functional competency may yield insight into targets that can enhance DPP4 driven improvements to HCT. We harvested BM from mice treated with the DPP4 inhibitor Diprotin A (DPA) and sorted immunophenotypically defined HSCs, multipotent progenitors (MPPs), common myeloid progenitors (CMPs), and granulocyte-macrophage progenitors (GMPs) for transcriptomic analysis. Linear modeling to examine differential gene expression controlling for cell type revealed that DPP4 inhibition gave strong enrichment of gene programs associated with mitochondrial function. Using a mitochondrial stress test and the Seahorse metabolic flux analyzer, we found that lineage depleted mouse BM cells treated *ex vivo* with DPA had lower basal oxygen consumption rates and spare respiratory capacity. These seemingly paradoxical data are consistent with reports that highly functional HSCs have higher mitochondrial mass but exhibit less mitochondrial activity. Thus, DPP4 inhibition may preserve high levels of functional competency by reducing cellular dependence on oxidative phosphorylation.

To examine DPP4 inhibition effects on individual subpopulations of HSCs/HPCs, we modeled each cell type separately for differential gene expression. We found that gene programs associated with neutrophil development were significantly enriched in HSCs and MPPs from mice treated with DPA, including genes *Elane*, *Lcn2*, *Prtn3* and *Ctsg/h*, concomitant with downregulation of platelet development programs in HSCs and GMPs from mice treated with DPA, including genes *Gp6/9*, *P2ry12* and *Clec1b*. This suggests that DPP4 drives improved engraftment in part by priming HSCs/HPCs for neutrophil recovery but does not enhance platelet development.

We then sought to target a platelet activating pathway in combination with DPP4 inhibition to improve HCT. Recipient mice were treated on days -2, -1, and 0 with 75 mg/kg DPA and day 0 with 50 μg/kg and days 1, 3, 5, and 7 with 25 μg/kg recombinant human Thrombopoietin (TPO), a platelet activating agonist. On day -1 mice were lethally irradiated and transplanted via tail vein with 2.5x10 ^{5} whole BM donor cells on day 0. Complete blood counts 2 weeks following transplantation showed that mice treated with DPA alone exhibited worse platelet recovery, in line with our transcriptomic data. However, mice treated with the combination of DPA and TPO exhibited significantly better platelet recovery than vehicle controls and DPA alone. Further, neutrophil recovery trended towards improved recovery in the combination treatment compared to the single agent treatments. Flow cytometry of the PB revealed no differences in BM homing or in donor PB chimerism one month following HCT in the combination treatment compared to single agents (though all treatments showed improved homing over vehicle controls), suggesting the primary effects were on early recovery and not on initial or long-term engraftment.

These data elucidate the DPP4 dependent transcriptome in the hematopoietic compartment, providing molecular insights into genes that drive enhanced functional competency of HSCs/HPCs. We also demonstrate that the DPP4 dependent transcriptome can reveal gene programs that can be targeted in combination to provide additive improvement on transplantation recovery.

Disclosures No relevant conflicts of interest to declare.

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