2020 - GPRASP FAMILY MEMBERS REGULATE MURINE HEMATOPOIETIC STEM CELL MIGRATION, HOMING AND NICHE RETENTION POST-TRANSPLANT VIA CONTROL OF CXCR4 STABILITY
Antonio Morales-Hernández, Ashley Chabot, Mahreen Ferdous, Shannon McKinley-Freeman
St Jude Children’s Research Hospital, Memphis, United States
Hematopoietic stem cell (HSC) transplantation (HSCT) represents the only curative therapy for most hematologic disease including leukemia. Recent studies suggest that “stress hematopoiesis”, including that which occurs post-HSCT, is subject to distinct regulation compared steady-state hematopoiesis. Better understanding the molecular mechanisms that regulate transplanted HSCs and their engraftment is necessary to improve HSCT. Thus, we seek to identify novel molecular regulators of HSCT. Our data reveal high expression of multiple GPRASPs (G-Protein Coupled Receptor Associated Sorting Proteins) (e.g. Gprasp1 and Gprasp2) in HSCs relative to downstream progenitors. ShRNA-mediated gene knock-down transplantation-based studies revealed that both GPRASP1 and GPRASP2 act as negative regulators of HSCT, showing 4-fold increase in peripheral blood and bone marrow reconstitution and significantly enhance survival and quiescence in HSCs ex vivo and after transplant. GPRASPs regulate the post-endosomal trafficking of GPCRs to the lysosome. CXCR4, a GPCR that regulates HSCs function, contains a putative GPRASP-binding motif in its C-terminus, implicating it as a possible target of GPRASP-mediated degradation. Indeed, Gprasp1 or Gprasp2 loss increased both total CXCR4 and cell membrane-localized CXCR4 in HSPCs. Consequently, Gprasp1 or Gprasp2 loss increased HSPC migration toward SDF-1. Further, Gprasp1 or Gprasp2 loss significantly enhanced acute homing and HSC niche retention post-transplant. These GPRASP knockdown effects disappear when Cxcr4 is genetically deleted. In sum, we report for the first time that multiple GPRASPs function as barriers to HSCT by effecting HSC migration, homing and niche retention via regulation of CXCR4 stability and localization.

2022 - IDENTIFICATION OF A RETINOIC ACID-DEPENDENT DEFINITIVE HEMATOPOIETIC PROGENITOR FROM HUMAN PLURIPOTENT STEM CELLS
Christopher Sturgeon1, Stephanie Laugh2, Carissa Dege2, Rebecca Scarfo3, Sara Maffioletti1, Samantha Morris3, Andrea Ditadi1, Christopher Sturgeon4
1Washington University in St. Louis, St. Louis, United States; 2Washington University in St. Louis, Saint Louis, United States; 3San Rafael Telethon Institute for Gene Therapy, Milan, Italy; 4Washington University, Saint Louis, United States
The generation of the hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs) is a major goal for regenerative medicine. In the embryo, HSCs derive from a HOXA+ population known as hemogenic endothelium (HE) in a retinoic acid (RA)-dependent manner. Using hPSCs, we have previously identified a KDR+cD35a-cXCR4- mesodermal population that gives rise to a clonally multipotent definitive HE. However, this lacks HSC-like capacity in the absence of exogenous transgenes, and is functionally unresponsive to RA treatment. Thus, the specification of a RA-dependent hematopoietic program from hPSCs has remained elusive. Through single cell RNAseq analyses, we identified that hPSC-derived hematopoietic mesoderm is actually comprised of three distinct KDR+ populations, distinguishable by CD235a and CXCX4 expression, prior to HE specification. Interestingly, CYP26A1, a RA degrading enzyme, was expressed in KDR+CD235a-CXCR4- mesoderm, and this was the only mesodermal population harboring definitive hematopoietic potential. In sharp contrast, KDR+CD235a-CXCR4+ mesoderm expressed ALDH1A2, a key enzyme in the synthesis of RA. When assessed for hematopoietic potential, this population completely lacked any hematopoietic activity. However, the brief, stage-specific application of retinol or all-trans retinoic acid to this CXCX4+ mesodermal population resulted in the robust specification of CD34+ HE with multi-lineage definitive erythroid, myeloid and lymphoid hematopoietic potential. Further, these CD34+ cells have fetal-like HOXA expression. Collectively, this represents the first ever characterization of stage-specific RA-dependent hPSC-derived definitive hematopoiesis, and its mesodermal progenitor. This novel insight into human hematopoietic development will ultimately provide the basis for the de novo specification of HSCs.

2021 - CHOLINERGIC INFLUENCE OF BONE MARROW VASCULAR MICROENVIRONMENT INFLUENCES HEMATOPOIETIC STEM CELL MOBILIZATION
Annas Al-sharea1, Man Kit Sam Lee2, Alexandra Whillas1, Michelle Flynn2, Lenny Strazukowski3, Christian Stolz2, Gerard Permes1, Joel Rimes3, Edwin Hawkins3, Prabakhar Nagareddy4, Louise Parson5, Andrew Murphy6
1Baker Heart and Diabetes Institute, Melbourne, Australia; 2Monash University, Melbourne, Australia; 3The Walter and Eliza Institute of Medical Research, Melbourne, Australia; 4Walter and Eliza Hall Institute of medical research, Melbourne, Australia; 5Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; 6University of Alabama at Birmingham, Birmingham, United States; 7St Vincent’s Institute, Melbourne, Australia
The regulation of HSC movement between the bone marrow (BM) microenvironment and the circulation remains an important clinical target in the setting of BM transplantation. The influence of parts of the nervous system, the sympathetic arm in particular, on BM niche function and mobilization have been investigated. However, the basal role of the cholinergic system in regulating HSC mobilisation is yet to be elucidated. We show that the cholinergic system and specifically the nicotinic -7 acetylcholine receptor (ChRNA7) mediates alterations in the structure and function of the BM sinusoidal endothelial cells as well as perivascular stromal cells (Lept+). Inhibition of this receptor with MLA leads to a loss of integrity in the BM sinusoidal structures along with a loss in perivascular niche stem cell factor production. Blocking ChRNA7 leads to mobilisation of functional HSCs without skewing that is typically present in G-CSF mobilization. This is suggested by single cell RNA sequencing of MLA mobilised peripheral HSCs that indicates a more balanced genetic line-age signature compared to those mobilised with G-CSF. In addition, administering MLA during G-CSF treatment leads to a synergistic effect, while stimulation of the ChRNA7 receptor with GTS-21 blunts G-CSF induced HSC mobilisation. These findings suggest a novel role for the peripheral cholinergic system in HSC mobilisation via niche cell alterations that provides a G-CSF independent alternative route for BM transplantation.

2023 - SIS-SEQ IDENTIFIES THE EARLIEST LINEAGE PRIMING REGULATORS OF DENDRITIC CELL FATE
Sara Tomei1, Layi Tian2, Jaring Schreuder3, Daniela Zalcstein4, Jessica Tran5, Nikołok Kocovska6, Shian Su7, Peter Diakami7, Melanie Bahlo8, Philip Hodgkin9, Matthew Ritchie10, Shalin Naik11
1The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; 2The Walter and Eliza Institute of Medical Research, Parkville, Australia; 3University of Alabama at Birmingham, Birmingham, United States; 4St Vincent’s Institute, Melbourne, Australia
Dendritic cells (DCs) are immune cells important for the detection and immunity against pathogens, self-antigens and cancer. They include three subtypes, conventional DC type 1 (cDC1), cDC type 2 (cDC2) and plasmacytoid DC (pDC) which all derive from a common hematopoietic stem cell progenitor population (HSPCs). Recent evidence has shown that this population is heterogenous for fate and not all HSPCs produce every DC subtype (Naik et al., 2007). To understand the earliest lineage priming regulators of DC fate, one would have to overcome the problem of the destructive nature of scRNA-seq, which makes it impossible for a single cell to be tested for both fate and its transcription profile. Here, we achieve this with a novel approach where a single progenitor cell is allowed to divide into a few cells, some of which are tested for fate while others tested for gene expression. By correlating clonal fate with gene expression, clone-by-clone, for 109 clones, we identified 490 genes that correlated with cDC1, cDC2 and/or pDC fate bias. All genes were tested in a pooled CRISPR/Cas9 screen in DC cultures where several novel genes were identified that regulated DC fate – the knockout of some genes enhanced numbers of particular DC subtypes, whereas others reduced numbers. This system could be used to study heterogeneity of fate at clonal level in other populations, including stem cells and cancer cells to reveal the transcriptional origin of fate decisions.