PROJECT DESCRIPTION

Project Title:

Preservation of endogenous low oxygen signaling facilitates hematopoietic cell phenotype, function, and clinical utility

Principal Investigator:

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Research Location:

Northeast Ohio Medical University College of Medicine
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Abstract:

Hematopoietic stem cells (HSC) are derived in the bone marrow (BM), give rise to all lineages of cells of the immune system throughout life, and can be transplanted to reconstitute the immune system. The bone marrow niche provides critical signals for hematopoietic stem and progenitor cell (HSC/HSPC) maintenance, self-renewal, and differentiation. Previous studies identified retaining HSC/HSPC in endogenous low Oxygen (low O_2, ~1-4% O_2) retains stem cell number, phenotype, and function, which is blunted when HSC/HSPC are exposed to ambient air (20% O_2). Despite this knowledge, most studies are performed in air, leaving endogenous signaling mechanisms unidentified, HSC/HSPC phenotype/function diminished, and transplant efficiency sub-optimal leading to high costs and patient complications. To address these knowledge gaps, we have generated and patented novel equipment (US-11633730-B2 Issued 04/2023 (O'Leary)) to isolate/sort/analyze and transplant HSC/HSPC under endogenous, continuous low O_2 conditions. Using this novel technology, we generated the first reference landscape of endogenous low O_2 HSC/HSPC phenotype/signaling/function. These studies identified important roles for multiple mechanistic pathways including but not limited to; serine peptidase (Dipeptidylpeptidase-4, DPP4) and cytosolic/mitochondrial Calcium (Ca^{2+}) in the optimal regulation of HSC/HSPC phenotype/function in low O_2, during health and disease states, and set the foundation for discovery of additional novel regulatory pathways. Retaining HSC/HSPC in low O_2 for optimal clinical utilization is technically challenging and brief exposure to ambient air ablates the low O_2 enhancement in numbers, phenotype, and function. Therefore, our goals for this study are to identify, and analyze, the differential regulation of endogenous low O_2 pathways to facilitate the pharmacologically mimicking of the low O_2 HSC/HSPC signaling, phenotype, and function, in air, enhancing transplant efficiency/utility.

Significance:

Hematopoietic cells are the only organ tissue that can be consistently transplanted with minimal or no risk to donor, autologously (self) or allogeneically (to another person), and result in reconstitution of the entire immune system, and cure of some diseases (bone marrow failure, sickle cell anemia, HIV with CCR5 mutated cells). The full potential of these cells has not been achieved due to a lack of understanding of signaling and function under endogenous low O_2 conditions. Manipulation of cells in air facilitates diminished numbers, and quality, of HSCs resulting in inefficient engraftment/complications, enhanced numbers of cells needed for transplant, requiring multiple donors for utilization of cord blood, and increased financial burden with most allogeneic transplants costing between $400,000-$800,000. Identification, and replication, of critical endogenous HSC signaling will facilitate enhanced phenotype, function, and interaction with the BM microenvironment resulting in enhanced cell utility/transplant efficiency, decreased cost, and diminished complications.

Goals and Objectives for the Research Project

Our goals for this project are to identify, and analyze, the differential regulation of endogenous low O_2 pathways to facilitate the pharmacologically mimicking of the low O_2 HSC/HSPC signaling, phenotype, and function, in air, enhancing transplant efficiency/utility.

Research Methods and Resources

Samples utilized will be isolated from animal models (C57 BLK/6 background mice that are control or have mutations to mimic human disease states, blood and bone marrow) as well as from primary patient samples (umbilical cord blood, peripheral blood of patients with and without hematologic disease). Phenotypic and functional studies will consist of flow cytometric analysis and sorting of cell populations (BD Melody, FACS Chorus, patented equipment), pharmacological and molecular analysis, generation of additional omics data (single cell RNA sequencing, proteomics etc.) replating studies to *in vitro* determine stem cell capabilities, and *in vivo* engrafting studies. High level statistical and bioinformatic analysis is completed in collaboration with additional expertise. Please see publications for in-depth overview and example details (PMID: 37051890, PMID: 28344320, PMID: 26073944).

Methods of Data Analysis

Flow cytometry and statistical data analysis will be performed using commercially available software (FLOWJO, SigmaPlot, & GraphPad). Based on the study design, many data will be analyzed using paired t-tests and repeated measures ANOVA.

Anticipated Findings

Our published and preliminary data suggest that combined pharmacological inhibition will enhance phenotype and signaling in HSC/HSPC populations, we anticipate these further studies will facilitate the identification of additional pathway interactions to modify and lead to determination of functional, clinically relevant, alterations.

STUDENT FELLOW TRAINING/MENTORING PLAN

Training Plan

All learners will participate in background literature review as well as required CITI training and CMU training in accordance with IACUC or IRB protocols. Resources in the form of webinars, lectures, and access to American Society of Hematology (ASH) education materials are made available for those that want additional insight. As Dr. O'Leary has a long standing history with ASH and their programs/awards, those interested in hematology will have support to help increase their interaction with ASH and their programs, if desired. Training is structured on an individual basis to ensure support of the trainee for their long-term overall goals as well as their lab goals. All trainees are taught through a "see one, do one, teach one" model and every member of the laboratory will cross-train in every function of the laboratory from sample collection to data acquisition and analysis to dissemination/write up of information under the instruction and supervision of Heather A. O'Leary Ph.D. and additional lab staff. Each member of the laboratory will have bi-weekly individual meetings in addition to group/lab meetings to discuss short/long term goals, current experimental data, intellectual scientific development, individual strengths and where the trainee needs additional support etc. Trainees will also participate in departmental and other research group meetings, seminar speakers etc. Trainees will be encouraged to assist our collaborators in any studies, at the discretion of those laboratories, and will be encouraged to explore the techniques and approaches employed by other faculty. Dr. O'Leary makes herself readily available for questions and consistent guidance as well as assists in learning data analysis, weekly data

analysis, generation of data figures, presentations, and practice for presentations. The lab goal is to have a fun, inclusive, safe, and productive environment for learning where people feel comfortable asking questions and are willing to work as a team to support each other. If desired, Dr. O'Leary is happy to connect you with former lab members to discuss the mentoring and laboratory environment.