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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Local Oxygen Dictates Hematopoietic Cell Growth and Function

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Functional hematopoietic stem (HSC) and progenitor cells (HPC) are responsible for the replenishment of blood/immune cells, are a source of treatment for diseases, and when dysregulated are a source of hematological neoplasia. To identify factors that impact HSC/HPC functional competency, we performed a transcriptomic screen which revealed that human cord blood (CB) HSCs/HPCs capable of early engraftment in an NSG mouse model of transplantation exhibited enriched expression of gene programs that vary with changes in oxygen (O_2) availability compared to non-transplanted input cells. This is physiologically and clinically relevant as HSCs/HPCs and diseased hematopoietic cells such as acute myeloid leukemia (AML) are exposed to O_2 tensions ranging from <1% to 21% as they function in various anatomical sites or are harvested for clinical applications. Thus, there are translational and practical rationales for understanding the growth and functional properties that hematopoietic cells exhibit across the range of O_2 they experience physiologically or artificially, which we examined here.
First we found significantly different frequencies of HSCs/HPCs and colony forming units (CFU) in human bone marrow (BM), mobilized peripheral blood (PB), and CB, which have varied O_2 tensions, suggesting O_2 availability may impact hematopoiesis. To directly examine this, human CB CD34+ cells were split and plated in CFU assays or expanded in growth stimulating conditions at 1% O_2, 3% O_2, 5% O_2, 14% O_2 (physiologic tensions), or 21% O_2 (processing tension) . HPCs expanded best and formed more differentiated CFUs in high physiologic O_2 tensions and extra physiologic O_2 tensions, while HSCs expanded better and more potent CFUs were formed in a mid-physiologic O_2 tension (5%). This suggests that O_2 dependent pathways are critical to HSC/HPC growth but there may be a "sweet spot" for early HSCs/HPCs that allows for proliferation while maintaining self-renewal and potency to maintain a pool of primitive cells, possibly mirroring *in vivo* O_2 responses. In *in vivo* functional HSC/HPC analyses, NSG mice transplanted with CB units expanded in physiologic O_2 exhibited significantly better early neutrophil recovery and long-term myeloid reconstitution compared to input, suggesting that growth and maintenance of HSC/ myeloid HPC functional competency is better balanced in physiologic O_2 compared to extra physiologic tensions. Platelet recovery and lymphoid reconstitution were significantly better in mice receiving uncultured control CB, suggesting expansion does not affect all HSC/HPC subpopulations equally. Mechanistically, cells grown in low O_2 tensions (1-3%) had higher frequencies of early HSCs/HPCs, significantly lower intracellular ROS levels, a significantly higher percentage of cells in cell cycle G0, and lower levels of phosphorylated γH2AX than those grown in higher O_2. These data suggest that while to some extent HSCs/HPCs require O_2 to proliferate, they also exhibit properties associated with exhaustion when maintained in higher O_2.
We next examined the effects varying O_2 tensions have on diseased hematopoietic cells using primary mouse AML cells or oncogene transformed human CB CD34+ cell lines. AML cells proliferated significantly more rapidly and formed more CFUs in higher O_2 tensions, suggesting that O_2 sensing pathways may be a dependency in AML that could affect disease progression as local O_2 tensions become perturbed. AML cells grown in lower O_2 exhibited lower resting levels of phosphorylated γH2AX (measured by flow cytometry) and lower O_2 consumption rates (measured by Seahorse metabolic flux analyzer), suggesting they are less susceptible to cellular stress. In line with this, AML cells maintained in low O_2 exhibited significantly better proliferation and recovery after treatment with the chemotherapeutic cytarabine compared to those in higher O_2 tensions. Thus, diseased cells growing in lower O_2 niches may be less susceptible to treatment and therefore may be a source of chemoresistance or relapse.
These data reveal important roles for O_2 in balancing hematopoietic cell growth with normal and diseased cell function. Manipulating O_2 tension or O_2 sensing pathways could be used to improve HSC/HPC expansion for transplantation or to treat disease. Transcriptomic studies of HSCs/HPCs and AML cells are ongoing to determine the O_2 sensing mechanisms that dictate cellular function.

Disclosures No relevant conflicts of interest to declare.

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