THE HARTWELL FOUNDATION

2021 Individual Biomedical Research Award

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Establishing a Physiologic Platform to Uncover Genetic Dependencies in Blood Cancers

Environmental factors influence growth and self-regulation of normal living cells, including maintenance of tissues and organ function. CRISPR, a revolutionary technology that enables the precise turn off (knockout) any one of nearly 20,0000 known human genes, makes it possible to evaluate how specific knockouts affect cancer cell characteristics like survival, growth, and vulnerability for treatment. However, a fundamental limitation to this approach is that CRISPR-based screens are routinely performed under cell growth conditions with little relevance to physiologic processes, in model systems that offer limited biological control over time and poorly reflect conditions in the human body. To address this problem, I developed a novel reagent, Human Plasma-Like Medium (HPLM), to sustain the growth of certain cells under nutrient conditions that closely simulate those found in the blood circulation. Based upon my discovery that growing cells in HPLM versus conventional media has widespread effects on genes that are most important for cell growth, I propose performing CRISPR-based screens on blood cancer cells cultured under tightly controlled and balanced "steady state" growth conditions using HPLM in a bioreactor model system. As a proof of concept, I will examine T-cell acute lymphoblastic leukemia (T-ALL), an aggressive blood cancer that affects white cells, particularly T lymphocytes. It accounts for 15% of the 4000 newly diagnosed childhood ALL cases in the US each year. Sadly, while survival rates for pediatric T-ALL have improved to nearly 80%, the intensive chemotherapy required for cure causes adverse side-effects for survivors and is ineffective for the 20% of children that relapse. My hypothesis is that CRISPR knockout screens of T-ALL cells made using HPLM, run under steady state conditions that accurately reflect those in the blood circulation, will reveal essential genes that cannot be identified by similar screens in a standard (batch) format. Using this platform, I will seek to identify new or hidden vulnerabilities in T lymphocytes and associated lymphoid stem cells that can be targeted with chemotherapy. I will perform CRISPR loss-of-function screens on genetically diverse T-ALL cell lines using a genome-wide single guide RNA library, comparing standard batch versus steady state culture formats. The strongest scoring conditionally essential candidate genes across T-ALL cell lines will be examined to delineate their contributions to gene essentiality, and a corresponding single-gene knockout cell line will be engineered. Metabolic profiling of cells and in vivo drug responses in mice will inform on the importance of high-priority essential genes for the growth of T-ALL. If I am successful, a promising new T-ALL therapeutic target(s) will be identified that would otherwise be overlooked by traditional CRISPR gene therapy. Clinical translation of such an advance has exciting potential to address the poor prognosis and quality of life for children diagnosed with this blood cancer.