Targeting physiologic energy metabolism for high fidelity patient-specific stem cell models of cardiac and neural disease

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Abstract

Human induced pluripotent stem cells (iPSCs) offer unparalleled potential to investigate human development, etiology of complex disease using patient cells, and as regenerative therapeutics for personalized medicine. However, clone-to-clone variability, poor differentiation capacity, and immaturity of hPSC-derived progeny may compromise their downstream applications. Traditional culture conditions do not recapitulate the *in vivo* stem cell niche, and we have observed that artificial levels of oxygen and metabolites lead to metabolic dysregulation that compromises hPSC developmental competence into neural and cardiac lineages. Therefore, **we hypothesize that recreating an *in vivo*-like metabolic environment will increase metabolic plasticity to augment iPSC differentiation and maturation capacity and better recapitulate tissue development.** Our collaborative preliminary data supports this hypothesis as physiologic oxygen and metabolites dramatically remodel central carbon metabolism and support developmentally-relevant lineage-specific differentiation in iPSC-derived organoids. Leveraging synergistic expertise in single cell RNA sequencing (Andrews, ASU) and stem cell metabolism (Folmes, MCA) and unique infrastructure for oxygen control, we will define how physiologic oxygen and metabolites mechanistically drive differentiation along neural and cardiac lineages in development, maturation into cells relevant to adult/aging tissues, and the impact this has on human disease modeling.

Directed neural and cardiac organoid differentiation will be temporally captured for single cell RNA sequencing to assay for transcriptional correspondence to tissue-specific primary reference datasets across development and adult tissues. While some metabolic information can be assessed from sequencing datasets, rapid metabolic changes will be functionally assayed using spent media and extracellular flux assays to evaluate temporal metabolic dynamics of living cells across lineages. Indeed, physiologic conditions in iPSCs dramatically remodel their metabolome, including major energy sources (glucose and glutamine), energy transfer (creatine, phosphocreatine), epigenetics (acetyl-CoA, s-adenosylmethionine), short chain acyl-carnitines, and uric acid, which have been linked to differentiation propensity and will serve as initial targets for analysis. The transcriptomic and metabolic data collected from these studies will be used to gain biological insights into tissue-specific developmental programs and establish an iPSC model system that more reliably recapitulates human development and disease progression. As a proof of concept and to provide insights into a currently intractable disease, we will probe a specific metabolically associated disorder of cardiac and neural development and homeostasis, Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes (MELAS), for which we have previously developed patient-derived iPSCs.

This **project aligns with the strategic priority of the Mayo Clinic and ASU Alliance for Health Care to optimize health and the human body**, by defining how physiologic nutrient supply regulates developmentally relevant tissue-specific differentiation of iPSCs for **accurate disease models and innovative treatments**. Additionally, metabolism is a highly druggable and pliable target for identification of potential therapeutics, which we can test in our improved models *in vitro*. Importantly, **both the heart and the brain do not have tissue-resident stem cells nor regenerate in humans**. Therefore, it is essential that we build higher fidelity models to work toward regenerative medicine applications. Support of this application will provide essential preliminary data for resubmission of scored, but not funded, collaborative applications to the NIH and CZI.