SYSTEMS-LEVEL DISCOVERY OF QUALITY ATTRIBUTES AND CANDIDATE PATHWAYS FOR OPTIMIZED PRODUCTION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Numerous protocols exist for differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes (CMs). Although these methods have improved in efficiency over the past decade, they remain highly variable in their resultant purities, not only among different source hPSC lines but also between batches in the same cell line. This substantial heterogeneity of hPSC-CM product outcomes points to poorly-understood, highly sensitive, and uncontrolled variables present within the overall process. Herein, we have undertaken a multi-omic discovery approach to identify key temporal differences in cell attributes between high- and low-purity hPSC-CM differentiations to provide systems-level insights into underlying mechanisms which drive these populations to divergent endpoints. Specifically, we are combining metabolomic, proteomic, lipidomic, and transcriptomic analyses collected throughout the differentiation process for high- and low-purity (as assessed by %cTnT+ via flow cytometry) differentiation batches. In addition to gaining fundamental insights into the underlying biology of the differentiation process, we are extending our analyses to 1) identify putative critical quality attributes for use in on- or at-line analytics for continuous process monitoring, 2) enhance process robustness through the development of protocols aimed at depressing off-target pathways and enhancing on-target ones, and 3) establish potential feedforward/feedback control schemes based on real-time analytics to respond to in-process intermediate quality attributes through rational adjustment of process parameters.

To date we have identified novel putative candidate quality attributes for process monitoring and cellular pathways which may be able to be modulated to augment process robustness in a scaled manufacturing context. Beyond standard single-omic analytical workflows, ongoing work is aimed at integrating these data for deepened insight, including functional integration with systems-scale modeling and high-dimensional machine-learning methodologies to extract dynamic relationships among variables over time.