Using multi-omic network analysis to identify cancer-relevant signaling pathways and novel biomarkers

Natalie M. Clark1, Michael A. Giillette1, Shankha Satpathy1, Steven A. Carr2, D. R. Mani3

1The Broad Institute of MIT and Harvard

While several driver mutations for multiple cancer types have been identified by large-scale genomic analyses, their functional involvement in known signaling pathways and therapeutic implications remain relatively unknown. Recently, efforts have been made to integrate proteomic and post-translational modification (PTM) data with genomic and transcriptomic data to improve our understanding of these identified mutations and ascertain novel candidate biomarkers. These proteogenomic data are rich in information which could be leveraged to identify the signaling pathways dysregulated by these driver mutations and suggest potential therapeutic targets.

Towards this goal, we leveraged a computational method named Spatio-temporal Clustering and Inference of Omics Networks (SC-ION), a regression-tree-based network inference algorithm which can identify differentially regulated signaling networks from multi-omics data. SC-ION can support inferences of various network types such as transcription factor-target, kinase-substrate, and gene product-metabolite regulations. SC-ION can indicate which regulatory edges arise from the incorporation of certain omics data to highlight, for example, the impact of PTMs versus the global proteome. The output from SC-ION can be integrated with other curated networks, such as protein-protein interaction networks, or used for independent downstream analyses, such as identification of novel regulators.

Here, we describe the application of SC-ION to proteogenomic datasets generated by the Clinical Proteomic Tumor Analysis Consortium (CPTAC), spanning multiple cancer types. SC-ION is one component in PANOPLY, a multi-faceted data analysis pipeline routinely utilized for CPTAC datasets. PANOPLY is a cloud-based platform for automated and reproducible proteogenomic data analysis. PANOPLY performs integrated analysis of multi-omic data, providing a quick and comprehensive baseline analysis for proteogenomic studies, enabling researchers to focus on disease-specific biology. We highlight key conclusions we can draw from the SC-ION-inferred networks and how these inferences can lend fresh insight into the biology of cancer.

100321, https://doi.org/10.1016/j.mcpro.2022.100328

Multi-Omics Driven Assessment of Pathobiology in a 3D-Bioprinted Aortic Valve Disease Model Compared to Native Valves and 2D Culture

Cassandra L. Clift1, Mark C. Blaser1, Willem Gerrits1, Tan Pham1, Mandy E. Turner1, Jason L. Andresen2, Owen S. Fenton3, Joshua M. Grolman4, Gabriuzio Buffolo1, David J. Mooney5,6,7, Jesper Hjortnaes3,4,8,9, Jochen D. Muehlschlegel10, Masanori Aikawa1,11, Robert Langer5,6,7, Elena Aikawa1,11

1Center for Interdis. Cardiovascular Sciences, Brigham & Women’s Hospital, Harvard Medical School, 2Wyss Institute for Biologically Inspired Engineering, Harvard University, 3Center for Excellence in Vascular Biology, Brigham and Women’s Hospital, Harvard Medical School, 5John A. Paulson School of Engineering and Applied Sciences, Harvard University, 6David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, 7Experimental Cardiology Laboratory, Department of Cardiology, University Medical Center Utrecht, 8Department of Chemical Engineering, Massachusetts Institute of Technology, 9Regenerative Medicine Center Utrecht, University Medical Center Utrecht, 10Dept. of Anesthesiology, Brigham & Women’s Hospital, Harvard Medical School

Introduction: Calcific aortic valve disease (CAVD) is associated with significant morbidity and mortality in the developed world. During CAVD progression, mechanosensitive valvular cells respond to fibrosis- and calcification-induced tissue stiffening, which further disrupts cellular-driven pathophysiology and valve biomechanics. Currently, no pharmacotherapeutics are available for CAVD, in part due to the lack of appropriate experimental models that recapitulate this complex biomechanical environment. Similarly, there are limited mass spectrometry-driven studies that adequately assess the complexity of novel engineered valvular model systems.

Methods: We developed a methacrylated gelatin (GeIMA) and hyaluronic acid (HAMA) 3D-bioprinted CAVD model that mimics biomechanical properties of the human AV’s disease-prone fibrosa layer (confirmed via nanindentation). Human valvular interstitial cells (VICs) were either plated via traditional 2D-monoculture or encapsulated in GeIMA/HAMA 3D-bioprinted models. Culture models were then treated with Normal Media, organic phosphate-rich Osteogenic Media or inorganic phosphate-rich Pro-calciifying Media, to induce calcification. Human CAVD valve leaflets (n=4 donors) were used to assess the relevance of the in vitro models to in vivo conditions. LC-MS/MS proteomics were performed via reverse-phase chromatography coupled to an orbitrap mass spectrometer (Orbitrap Exploris 480) via data-dependent acquisition. Serial multi-enzymatic digestions allowed for the multi-omics analysis of the tryptic cellular proteome, along with the extracellular matrisome, vesiculome, and n-glycome, using collagenase type III, trypsin, and PNGaseF, respectively.

Preliminary Results: Cellular proteomics identified over 2500 proteins. Proteins enriched in the 2D system were enriched in biological processes of actin organization and platelet aggregation, while the 3D-bioprinted model proteome identified enrichment of O- and N-glycosylation, glycosaminoglycan biosynthesis, and extracellular-matrix organization. 3D Cellular proteomics identified enzymatic regulators of glycosylation, informing downstream PNGaseF-elucidated N-glycome composition. Extracellular Vesiculomics identified over 200 proteins, including 14 vesicle markers, with 88% overlap with the cellular proteome. Additionally, collagenase-targeted extracellular-matrisomics probed site-specificity and abundance of several post-translational modifications of collagen subtypes, including hydroxyproline and hydroxylysine.

Novelty: This study presents a novel 3D-bioprinted 96-well plate model of CAVD suitable for high-throughput drug discovery, sheds light on the impact of culture dimensionality on in vitro models of valvular disease, and positions multi-omics as a novel technique for the design and assessment of bioengineered model systems.

100321, https://doi.org/10.1016/j.mcpro.2022.100329