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Spatial Phosphoproteomic Profiling Reveals Regional Functional Heterogeneity in the Murine Heart

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Phosphorylation mediated signaling is fundamental to cardiac function. However, the dynamic signaling patterns across different spatial regions of the heart have been inadequately explored. This is mainly because of the technical difficulties in analyzing tiny tissue samples with the required depth and sensitivity. To tackle these challenges, we developed an optimized TiO_2 based micropipette tip method for in-depth phosphoproteomics. This methodology showcases outstanding sensitivity, identifying 12,173 class I phosphosites from 10 µg HeLa peptides, and also offers high reproducibility.

We then utilized this advanced technique to study spatially defined regions of the mouse heart. Through laser-capture microdissection, we isolated seven specific anatomical areas: the left atrium (LA), right atrium (RA), left ventricle (LV), right ventricle (RV), interventricular septum (IVS), apex (APEX), and aortic valve (AV). For each region, we were able to quantify 1,000-2,000 phosphosites. Principal component analysis revealed distinct phosphoproteomic signatures that cluster according to anatomical positions, providing higher resolution differentiation than proteomic profiling. Functional enrichment analysis further unveiled region-specific phosphorylation patterns. The APEX and ventricular regions were characterized by phosphorylation signatures associated with the contractile machinery. In the AV tissue, proteins related to cell junctions and polarity were significantly enriched. From a metabolic perspective, the LV exhibited phosphorylation patterns closely tied to energy metabolism, while the LA showed enrichment in RNA processing pathways. Phosphoproteins in the RA were predominantly involved in cellular component biogenesis and chromatin organization.

This spatially resolved atlas establishes a molecular foundation for investigating region-specific cardiac pathologies and advances understanding of post-translational cardiac heterogeneity.

User consent

yes

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