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Spatial Phosphoproteomic Profiling of Murine Heart Reveals Region-Specific Functions via TiO_2 Enrichment Optimized for Laser-Capture Microdissected Samples

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Phosphorylation-mediated signaling dynamics across spatially distinct cardiac regions remain poorly understood due to limited technical capacity for deep and sensitive analysis of minute samples. Here, we present an optimized TiO_2-based micropipette tip method for deep phosphoproteomics, achieving high sensitivity (12,117 class I phosphosites from only 10 μg HeLa peptides) and reproducibility. Applying this to laser-capture microdissected mice myocardial regions, i.e. left/right atria (LA, RA), left/right ventricles (LV, RV), interventricular septum (IVS), apex (APEX), and aortic valve (AV), we quantified 1,000–2,000 class I phosphosites per region (e.g., 1,050 in AV, which has an area of only 0.2 mm^2). Principal component analysis revealed distinct phosphoproteomic clustering aligned with anatomical positions, surpassing proteomic resolution. Functional enrichment uncovered region-specific functions: APEX and ventricles exhibited phosphorylation signatures linked to muscle contraction, while AV was enriched in cell junction and polarity. Metabolically, the LV demonstrated phos-phorylation patterns linked to energy metabolism, whereas LA showed enrichment in RNA processing. RA was pertinent to cellular component biogenesis and chromatin organization. This spatially resolved phosphoproteomic atlas elucidates func-tional specialization across cardiac subregions, establishing a molecular foundation for investing region-specific cardiac pa-thologies. Our approach addresses critical technical limitations in low-input phosphoproteomics while advancing under-standing of cardiac spatial heterogeneity at the post-translational level.

User consent

yes

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