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A BioID-derived proximity interactome for Protein Phosphatase PP2A

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Background: Protein Phosphatase 2A (PP2A) is an important and ubiquitously expressed serine threonine phosphatase in brain. Structurally, it is composed of catalytic (C), scaffold (A) and regulatory (B) subunits. It plays a critical role in regulation of many cellular processes and has been implicated in various neurological disorders including major depressive disorder (MDD). MDD is the most common mental disorder affecting up to 17% of the population in the USA. To date, limited information is available related to the PP2A interactome. To dissect underlying mechanisms of PP2A function, we employed proximity-dependent biotin-identification (Bio-ID) to identify the PP2A interactome.

Methods: To initially capture PP2A interactors and optimize workflows, HEK 293 T cells were transfected with a PP2A-A subunit-BirA*-Flag construct. After 8 hours, growth media was supplemented with 50 µM biotin and cultured further 16 h. After 24 hours, cells were lysed and subjected to acetone precipitation to remove interfering reagents. Tryptic peptides were desalted and enriched using anti-biotin antibody. The peptides were subjected to LC-MS/MS and the spectra were searched with variable modifications (+226.07 Da) at peptide N termini and lysine. The identified proteins were further analyzed for PPI's and pathways.

Results: A robust and sensitive method generated a total of 1438 proteins and 396 biotin-K and 4 biotin-N terminal sites from 260 biotinylated proteins. PPI network analysis generated a high confidence interactome of 199 nodes with high interaction score. Further, pathway analysis showed involvement of PP2A interactors in regulation of phosphoprotein phosphatase activity, regulation of cytoskeleton organization, cell adhesion, protein phosphatase 2A binding, actin filament binding and calmodulin binding.

Conclusion: The systematic analysis of the PP2A interactome revealed high density interactions which allows more precise understanding of the phosphatase function. The validation of a few potential interactors is required to better understand the biological significance of the PP2A interactome at a molecular and cellular level. In the future, examination of the PP2A interactome(s) associated with MDD related mutations in cell and animal models may shed light on the pathophysiology of MDD and potentially help to identify new targets for treatment of this highly disabling disorder.

Keywords: Proximity Labeling, Bio-ID, LC-MS-MS, PPI's

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Proteomics of spatially identified tissues in whole organs

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Spatial molecular profiling of complex tissues is essential to investigate cellular function in physiological and pathological states. However, methods for molecular analysis of biological specimens imaged in 3D as a whole are lacking. Here, we present DISCO-MS, a technology combining whole-organ imaging, deep learning-based image analysis, and ultra-high sensitivity mass spectrometry. DISCO-MS yielded qualitative and quantitative proteomics data indistinguishable from uncleared samples in both rodent and human tissues. Using DISCO-MS, we investigated microglia activation locally along axonal tracts after brain injury and revealed known and novel biomarkers. Furthermore, we identified initial individual amyloid-beta plaques in the brains of a young familial Alzheimer's disease mouse model, characterized the core proteome of these aggregates, and highlighted their compositional heterogeneity. Thus, DISCO-MS enables quantitative, unbiased proteome analysis of target tissues following unbiased imaging of entire organs, providing new diagnostic and therapeutic opportunities for complex diseases, including neurodegeneration.

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