Identification and characterization of alterations in the DARPP-32 protein interactome in rat striatal tissue following cocaine treatment

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Background: Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32), is an abundant protein in dopaminoceptive striatal medium spiny neurons, which act as a multifunctional regulator of several protein kinases and phosphatases and plays critical roles in integrating electrophysiological, biochemical, and behavioral responses in the actions of multiple drugs of abuse. DARPP-32 is therefore a signaling hub protein, interacting with multiple proteins in concert, resulting in the regulation of various signaling pathways. Although the interaction of individual striatal proteins with DARPP-32 and its phosphorylation states have been well characterized, global profiling of the DARPP-32 protein interactome has not been carried out.

Method: We used an in situ biotinylation by antibody recognition (BAR) based proximity labeling approach to spatially resolve the proximal potential protein interactors of DARPP-32 in rat striatal tissue slices. The differential interactome of DARPP-32 in striatal tissue after treatment with cocaine was also identified. Protein interactions were validated using co-immunoprecipitation and immunoblot analysis.

Results and Discussion: DARPP-32 proximal proteins (1952 proteins) identified by BAR proximity labeling in striatal tissue were found to be involved in many aspects of neuronal function including protein serine/threonine kinase activity, cAMP-dependent protein kinase activity, regulation of phosphorylation and dephosphorylation activity. Co-immunoprecipitation validation using recombinant DARPP-32 and striatal lysate identified 40% of the DARPP-32 Co-IP proteins to be present in the BAR dataset. Immunofluorescence labeling of DARPP-32 in striatal tissues of rats treated with cocaine showed DARPP-32 was predominantly nuclear localized. Comparison of the intensities of DARPP-32 biotinylated proteins in rats treated with saline/cocaine showed that 32 proteins were significantly differentially biotinylated (>1.5 fold) in rats exposed to cocaine for 1.5 hour and 33 proteins for 24-hour.

Conclusion: The DARPP-32 interactome identified using proximity labeling provides new insights into the functional roles of this protein in interactive signaling pathways related to cocaine addiction in dopaminoceptive striatal neurons. This study provides support for using the BAR proximity labeling approach to explore the dynamic changes due to protein localization such as cytonuclear trafficking in response to molecular stimuli.


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The term autophagy (from the Ancient Greek autóphagos, meaning self-eating) describes a process in which cytoplasmic cargo is sequestered inside double-membraned vesicles and subsequently degraded by lysosomal enzymes. Autophagy plays a central role in many cellular processes but has also been extensively implicated in the pathology of many diseases, including in cancer. However, the role of autophagy in cancer appears complex, paradoxical, context-dependent and cancer specific and the exact nature of its role is yet to be elucidated. Although initially thought of as a bulk degradation system, it is now clear that autophagy can selectively sequester cargo. This project aims to study autophagy’s role in cancer by developing new assays that will enable extensive profiling of the autophagy degradome. We are developing novel peptide autophagy inhibitors derived from Phytophthora infestans virulence factors, combined with genetic tools and mass spectrometry based quantitative proteomic techniques. These optimised protocols will be expanded in a pan-cancer fashion to establish high confidence, cancer-specific autophagy cargo, which has the potential to explain the differential role of autophagy between cancers and identify cancer-specific therapeutic vulnerabilities.

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