3.3 Pirating Biology to Probe and Attack the Surfacome in Cancer

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The cell surface proteome is a major hub for cellular communication and a primary source of drug targets, especially for biologics. Identifying how the surfacome changes in cancer is a central challenge for identifying and targeting disease associated proteins. We have used chemical methods and engineered proteins to facilitate identification of membrane proteins, both native and post-translationally modified versions, that change with oncogene transformation and or hypoxia a characteristic of the tumor microenvironment. We have developed a simple promiscuous biotinylator which tethers HRP to the membrane allowing more sensitive labeling of cell surface proteins. Proteolysis is also a hallmark of cancer reflecting metastasis and tumor maintenance, yet we have little understanding of the proteolytic targets. We have engineered a peptide ligase, subtiligase, that can be tethered to the membrane and used to tag proteolytic events on the cell surface. We have used this to identify proteolytic neo-epitopes in cancer beginning with the systematic identification of cell surface proteolytic events. We then target proteins either upregulated, proteolyzed or both with recombinant antibodies derived by phage display to be used as validation tools and potential therapeutic leads.

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3.4 Multiplexed proximity biotinylation to uncover the axon initial segment proteome

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Neuronal action potentials are generated at axon initial segments (AIS). AIS also maintain neuronal polarity by regulating the differential trafficking and distribution of proteins, transport vesicles, and organelles. However, the mechanisms regulating these functions at the AIS remain poorly understood. Furthermore, due to the dense and robust cytoskeleton at the AIS, proteins found here are refractory to traditional approaches for affinity purification and proteomics. To overcome these limitations, we applied two separate proximity biotinylation strategies to identify AIS proteins. We used BioID-based proximity biotinylation to identify the cytoplasmic AIS proteome, and biotinylation by antibody recognition to define the AIS surface proteome. We validated many of the identified proteins using homology independent, Cas9-dependent genome editing to tag endogenous proteins, determine their localizations, and functions.

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