O-10.4 Short talk

Single-Molecule and Multiscale Fluorescence Imaging to Investigate PD-L1 Dynamics and its Association with Lipid Rafts in Non-Small Cell Lung Cancer Cells

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The membrane protein PD-L1 plays a pivotal role in tumors and is a key target for immunotherapy. While its immunosuppressive function in the PD-1/PD-L1 checkpoint is well characterized, its nanoscale regulation remains unclear and is likely influenced by spatial organization and diffusion dynamics. We employed super-resolution and single-molecule fluorescence techniques to investigate PD-L1 organization and mobility in non-small cell lung cancer (NSCLC) cells. Single-Molecule Localization Microscopy (SMLM) revealed PD-L1 clustering (~30-40 nm) within cholesterol-enriched membrane rafts, confirmed by colocalization with key raft markers. Fluorescence Correlation Spectroscopy (FCS) and spot variation-STED-FCS in live cells uncovered confined diffusion, typical of raft-associated proteins, indicating a tightly regulated mobility. PD-L1 raft association may influence immune checkpoint activity, impacting immune evasion and therapeutic strategies. Additionally, a non-clustered PD-L1 pool may have alternative functions. To validate our findings, we have performing PALM to compare SMLM data from antibody-based and genetically encoded fluorescence labeling. By integrating multiscale fluorescence imaging with single-molecule biophysics, this study provides novel insights into PD-L1 spatial organization and its role in NSCLC pathophysiology and immunotherapy.