Hybrid methods reveal the structural architecture of PTPN3-p38γ active-state complex

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Statement of the Problem: The mitogen-activated protein kinase p38γ (also known as MAPK12) and its specific phosphatase PTPN3 (also known as PTPH1) cooperate to promote Ras-induced oncogenesis in human colorectal cancer. Comprehensive structural information on the PTPN3-p38γ interaction is critical for structure-based drug design as a new means in anticancer therapy.

Methodology & Theoretical Orientation: In order to obtain the architecture features of PTPN3-p38γ active-state complex, a hybrid method combining small-angle x-ray scattering (SAXS), chemical cross-linking coupled to mass spectrometry (CX-MS), hydrogen deuterium exchange mass spectrometry (HDX-MS), and x-ray crystallography were adopted.

Findings: To build the molecular architecture of PTPN3-p38γ active-state complex, the phosphatase domain of PTPN3 in its substrate trapping mutant form was used to interact with the phosphorylated p38γ in vitro. Isothermal titration calorimetry (iTC) analysis showed that PTPN3 binds to phosphorylated p38γ in a submicromolar affinity, suggesting the formation of a stable complex. CX-MS analysis unraveled the close proximity between the catalytic site of PTPN3 and the activation loop of phospho-p38γ. HDX-MS results further demonstrated that the glutamic acid-containing loop (E-loop) and the phosphotyrosine recognition loop (pY loop) of PTPN3 play a critical role in recruiting p38γ as a substrate during catalysis. Preliminary crystals of PTPN3-p38γ active-state complex were obtained and the microseeding technique was applied to optimize the crystal formation.

Conclusion & Significance: Atomic structure of PTPN3-p38γ active-state complex may reveal the molecular features for the design of new drug against the progression of colorectal cancer.

Figure 1: HDX-MS results suggest a critical role of the E-Loop and the pY loop of PTPN3 in the formation of the active state complex with p38γ.

Recent Publications


\textbf{Biography}

Shu-Fang Hsu has her expertise in cell biology focusing on tyrosine phosphorylation-dependent cell signaling which coordinated by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). PTPs comprise a superfamily of enzymes that control a diverse array of signal transduction pathways, therefore exert their biological functions including tumorigenicity. Her current interest is crystal structure study on PTPN3-p38γ complex for structure-based drug design as an anticancer therapy for colon cancer.