New Directions for Therapeutic Treatment of Colorectal Cancer

*This report features the work of Kai-En Chen, Tzu-Ching Meng, Andrew H.-J. Wang and their co-workers published in Sci. Signal. **7**, ra98 (2014).*

The Ras signaling cascade has long been considered an attractive therapeutic target for colorectal cancer (CRC). Among the modules in this signaling pathway, p38γ and its specific phosphatase PTPN3 are known to be the key regulator responsible for Ras-mediated oncogenic activity. Recent attention has been drawn to the PDZ-mediated PTPN3-p38γ complex, which is known to be a novel target for Ras-dependent malignancies, but the dynamic nature of the phosphatase-kinase interactions have so far resisted efforts by structural biologists to explore the detailed molecular insight of drug targets in this important group. In 2014, a research team led by Andrew H.-J. Wang and Tzu-Ching Meng from Academia Sinica determined this complex with a hybrid method combining X-ray crystallography, small-angle X-ray scattering (SAXS) and chemical cross-linking coupled with mass spectrometry (CX-MS). The structure demonstrated the formation of an active-state complex and the unique

regulatory role of the PDZ domain of PTPN3.

Mitgoen-activated protein kinases (MAPK) are composed of four isoforms—p38α, p38β, p38γ and p38δ. p38γ differs from other p38 MAPK by the presence of a C-terminal ETPL motif,[1] which binds to the PDZ domain in interacting proteins. Previous work has shown that p38γ and its specific phosphatase PTPN3 (PTPH1) cooperate through the PDZ domain to promote Ras transformation in human cancers.[2] To investigate the molecular basis of the interaction between PTPN3 and p38γ, the research team used advanced X-ray techniques in the TLS, including protein crystallography beamline **BL15A1** and small and wide-angle X-ray scattering beamline **BL23A1**.

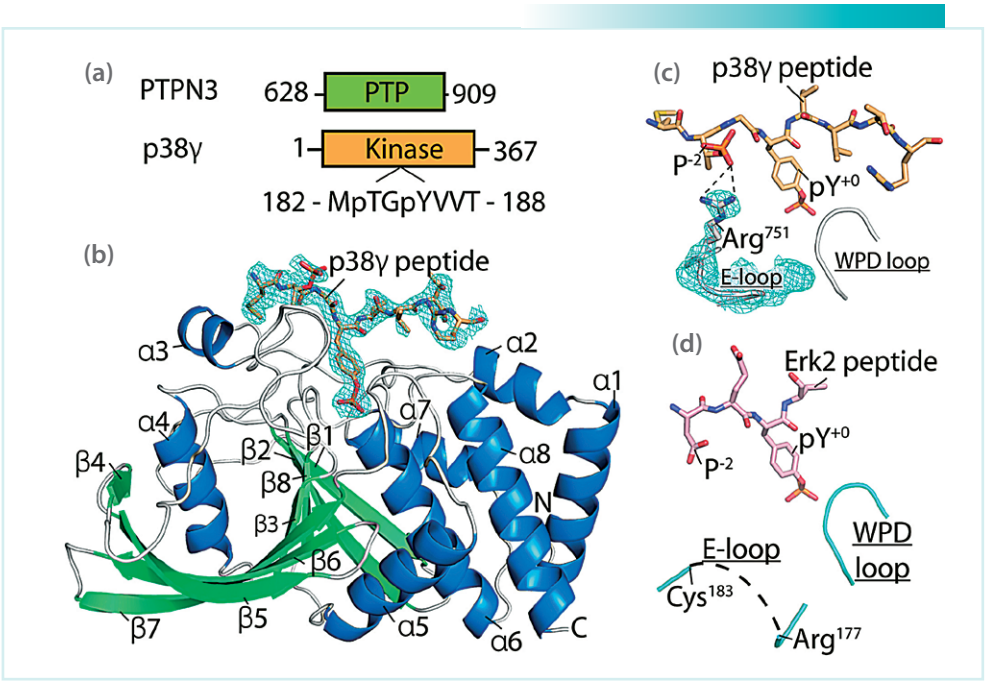

Fig. 1: Crystal structure of PTPN3 PTP domain D811A/C842S mutant in a complex with dually phosphorylated peptide p38γ. (a) Schematic showing the constructs of PTPN3 and p38γ. (b) Representation of the complex structure (PDB ID: 4QUM). (c) Active site of the complex structure highlights the interaction between Arg^{751} and $pThr^{183}$ (P^{-2}). (d) Active site of HePTP in the complex with the Erk2 mimetic peptide (PDB ID: 3D44). The electron density shown corresponds to a simulated-annealing OIMT F_o-F_c map contoured at 3σ. (Modified from Ref. 4)

In this work, the crystal structure of the PTPN3 PTP catalytic domain in a complex with dually phosphorylated peptide p38γ that mimics the TxY motif of the activation loop (Fig. 1(a)) was determined at 2.5-Å resolution. The structure revealed a monomer in the asymmetric unit with p38γ phosphopeptide clearly visible in the complex structure (Fig. 1(b)). According to Figs. 1(c) and 1(d), the unique feature in this complex structure is the salt bridge interaction between the phosphate oxygen of P^{-2}($pThr^{183}$ of p38γ) and nitrogen atoms of the guanidine group of Arg^{751} in the E-loop of PTPN3. In comparison with the structure of HePTP in a complex with a phosphorylated Erk2 peptide, such a salt bridge does not exist. Together with kinetic and mutagenesis data, this work indicates that PTPN3 prefers to recognize dually phosphorylated ($pThr^{183}$-Gly-$pTyr^{185}$) rather than mono-phosphorylated (Thr-Gly-$pTyr^{185}$) p38γ as a substrate.

To extend these findings, the research team applied SAXS to investigate whether PTPN3 and p38γ protein-protein complex in solution can support an active-state binding mode. As shown in Fig. 2(a), the pair distance-distribution plot indicated that the complex had a globular shape in solution, with D_{max} = 91 Å and R_g = 28.5 Å. The structure *ab initio* of the complex, calculated with DAMMIN, also revealed a compact shape similar to the active-state envelope of PTP-MPAK complexes published previously[3] (Fig. 2(b)). Using the FoXS calculation, the research team found that the active site pocket of PTPN3 was in close proximity to the activation loop of protein p38γ (Fig. 2(c)). The agreement between solution and crystal structure indicates that the active-state complex is the dominant form when PTPN3 recognizes p38γ.

Next, the research team applied the CX-MS ap-

proach to map the PDZ domain arrangement in the active-state complex. As shown in Fig. 3(a), three lysine residues (Lys^{520}, Lys^{526} and Lys^{532}) of the PDZ domain of PTPN3 were cross-linked with either Lys^{352} or Lys^{363} located on the C-terminal ETPL motif of dually phosphorylated p38γ protein.

In the absence of p38γ, the PDZ and PTP domains of PTPN3 form a unique intramolecular cross-linking that was not detected in the active-state complex. According to the distance restraint of the BS3 cross-linker, the PDZ and PTP domains are believed to form a compact conformation in the absence of p38γ (Figs. 3(b) and 3(c)). Together, the findings from this work indicate two new directions for a rational drug design to target human cancers, especially CRC, in which PTPN3 is highly expressed and expected to promote Ras oncogenic signaling through dephosphorylation of p38γ.

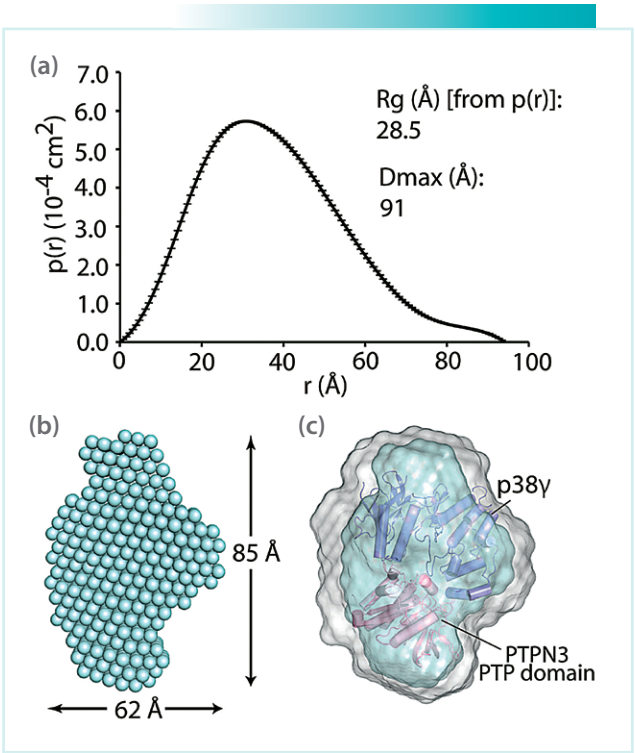

Fig. 2: SAXS data and model of the PTPN3-p38γ complex *ab initio* (a) Pair distance-distribution plots derived from the experimental scattering data using GNOM. (b) Probable shape of the complex obtained with an averaging and filtering process from program DAMAVER. (c) Envelope *ab initio* overlaid with the optimized models of the complex calculated from FoXS. (Modified from Ref. 4)

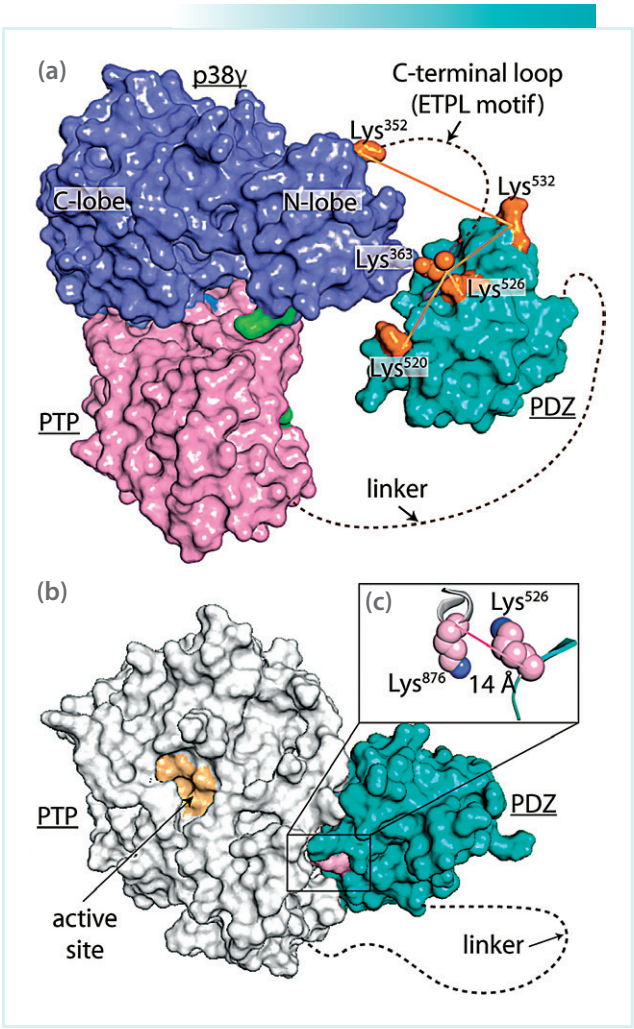

Fig. 3: CX-MS based assembly of the PTPN3 - p38γ complex. (a) Complex model with the PDZ domain attached near the N-lobe of p38γ. (b) Model structure of PTPN3 to show the close proximity of the PDZ and PTP domains. (c) Enlarged view showing the cross-linking pair. (Modified from Ref. 4)

References

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