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第43回日本免疫学会学術集会　テクニカルセミナー

第1日目12月10日(水) 12:00-13:00

国立京都国際会館　J会場(Room C-2)

演題

「Immunological tools to aid recombinant protein production and analysis」

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Peptide-based epitope tagging technology is universally used in nearly all kind of research projects that involve biochemical characterization of a target protein, but not many systems are fully compatible with purification purpose. By utilizing an anti-human podoplanin antibody NZ-1, we constructed a novel epitope tag system. NZ-1 possesses exceptionally high affinity toward a dodecapeptide (GVAMPGAEDDVV) dubbed “PA tag”, with a characteristic slow dissociation kinetics [1]. Because of its high affinity, PA-tagged proteins in a dilute sample can be captured by immobilized NZ-1 resin in a near complete fashion and eluted by a solution of free PA peptide. This enabled efficient one-step purification of various proteins including soluble and membrane proteins expressed in mammalian cells. Mild regeneration condition of the peptide-bound antibody ensures repeated use of the antibody resin, indicating a cost-efficient nature of the system. We have applied this purification system to over 20 target proteins and have succeeded in crystallizing several glycoproteins and receptor ectodomains with relatively short turnaround time of 1~2 months/project. Next we have determined the X-ray crystal structure of the NZ-1 Fab fragment bound by the PA tag peptide, and deduced precise binding mechanism at atomic resolution. Within the binding pocket of NZ-1, the PA peptide assumed a tight β-turn conformation, with the Pro-Gly sequence at the tip of the hairpin deeply inserted in the antigen binding groove. In other words, NZ-1 recognizes the middle part of the tag with little contribution from the distal N- and C-terminal parts. In fact, PA tag can be inserted into a tip of turn or in the middle of a loop region of a protein, enabling the site-specific fluorescent labeling of a receptor. As it is generally difficult to graft linear epitope tag in a structured protein domain, the PA tag system may provide unique opportunity to attach purification/labeling handle to a target protein.

[1] Fujii Y *et al*. *Protein Expr Purif*, 95:240-7 (2014)

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