Structure-based integrated approach for analysis of the GTP metabolism

Toshiya Senda[1], Koh Takeuchi[2], Atsuo Sasaki[3]

1 *Structural Biology Research Center, Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK)* **2** *Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology* **3** *Division of Hematology and Oncology, Department of Internal Medicine, University of Cincinnati College of Medicine*

Metabolic pathways in the organism form a large and multi-domain network involving numerous biochemical and physicochemical processes. Furthermore, these processes are regulated by intra- and inter-cellular signaling and gene expression. Since the structure of the metabolic network is quite complex, it requires specific means to analyze a relationship between cause and effect of metabolic processes, particularly in the study of metabolic disorders. To tackle this difficult problem, we have utilized a structure-based integrated approach. Here, we would like to present an example of this approach for studying the GTP metabolism and GTP-related metabolic disorders. In this study, we discovered a cellular GTP sensor [1] and have been opening a new field in the GTP metabolism. Initially, we identified a candidate of a GTP sensor, PI5P4Kβ, using biochemical methods, revealing that PI5P4Kβ is a unique kinase that can utilize GTP and ATP for its catalytic reaction with significant preference to GTP. Then, we examined biological functions of PI5P4Kβ using a structural reverse genetics approach [2], in which a mutant PI5P4Kβ was designed [1, 3] to remove one specific *biochemical* function, the GTP-dependent kinase activity. On the basis of crystal structures of PI5P4Kβ, we designed a FL mutant (Phe205Leu) that lacks the GTP-dependent enzyme activity without changing ATP-dependent activity and prepared a cell line harboring the FL mutant gene. We then demonstrated that PI5P4Kβ is a cellular GTP sensor by biological experiments using the FL and WT (wild type) cell lines. These cell lines were further utilized to explore signaling pathways of PI5P4Kβ. We have collected transcriptome data of these cell lines, and informatics analysis on the obtained transcriptome data is revealing relevant cellular processes. The 3D-structural data of PI5P4Kβ is also utilized to design inhibitors of PI5P4Kβ, which, in turn, are utilized as a tool for analyzing signaling pathways of PI5P4Kβ. In addition, a series of mutant enzymes with different enzyme activities would be useful for the analysis of signaling pathway. Since evolutionarily related PI5P4Kβ would show different enzyme activity and substrate specificity, we have purified several evolutionarily related PI5P4Kβ and initiated biochemical and structural studies.

In the structure-based integrated approach, crystal structure analysis of target proteins is crucial, therefore, techniques for reliable structure determination are required. Techniques for reliable structure determination, native SAD phasing, automated data collection, and methods of crystal engineering, will also be discussed in the presentation.

[References]
[1] Sumita, Lo, Takeuchi *et al*. ***Mol. Cell*** 21: 187-198 (2016). [2] Takeuchi et al. ***FEBS J***, 283: 3556-3562 (2016). [3] Senda et al. ***Cryst. Growth Des.*** 16: 1565-1571(2016).